LIFE SCIENCE

TCI

Antibodies and Related Reagents

Antibodies and Related Reagents

Antibodies and Related Reagents

Products for Labeling

Products for Cell Biology

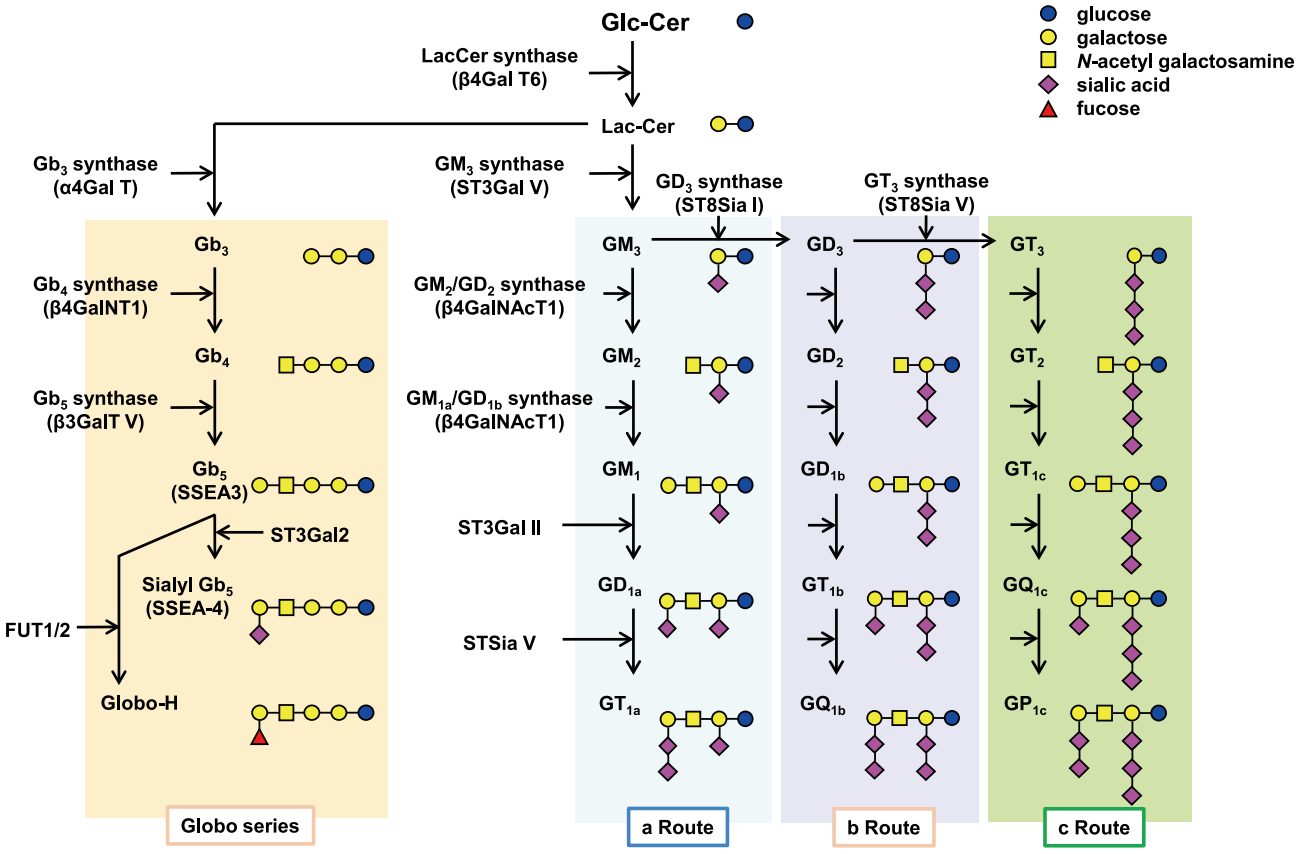
Enzyme Substrates

Other Products

Carbohydrate chains are called the third life chain following the protein and the nucleic acid and are one of the most important issues in the post genome research. Most carbohydrate chains attach to lipids or proteins and occur in the form of glycoproteins or glycolipids (*N*-glycan, *O*-glycan, proteoglycans and others). Carbohydrate chains are known to be expressed on brain, nerve, cancer, and endothelial cells. Some carbohydrate chains are known to relate to diseases (e.g., cancer, Alzheimer's disease, Guillain-Barré syndrome, Lysosome syndrome such as Fabry disease, gangliosidosis), differentiation and development (iPS/ES cells). Seasonal influenza viruses, annual epidemics that peak during winter, cause infection via cell-surface glycans. Anti-influenza virus drugs are structural mimics of sialic acid, because neuraminidase is a sialic acid hydrolase that is essential for the release of progeny virus particles from the surface of an infected cell.
Anti-carbohydrate antibodies can recognize glycolipids or glycoproteins. These antibodies can be used for immunohistochemistry, cell-staining, inhibition assay for cell adhesion, flow cytometry, ELISA, TLC-immunostaining and other methods.

Anti-Glycolipid Antibodies

Ganglioside/Globoside biosynthetic pathway

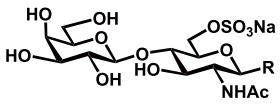


Gangliosides/globosides are cell surface glycosphingolipids composed of glycan and ceramide that play important functional roles in intercellular recognition, cell adhesion, and signal transduction. The carbohydrate moiety of gangliosides/globosides is synthesized in the Golgi apparatus via the sequential action of several glycosyltransferases. Changes in the expression patterns of gangliosides/globosides in development and disease are largely associated with changes in the expression of these glycosyltransferases, which are spatiotemporally regulated at both the transcriptional and post-translational levels. TCI offers antibody products useful for the detection of these glycolipids.

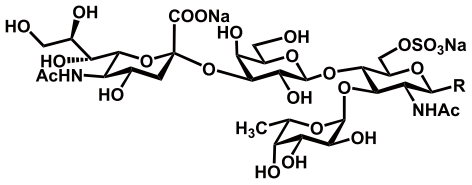
Product Name	Isotype	Size	Product Number
Anti-GM_1 Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2505]
Anti-GM_2 Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2576]
Anti-GM_3 Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2582]
Anti-GD_{1a} Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2507]
Anti-GD_{1b} Monoclonal Antibody	Mouse IgG3	0.1mg/vial	[A2508]
Anti-GD_2 Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A3338]
Anti-GD_3 Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2580]
Anti-GT_{1a} Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2702]
Anti-GT_{1b} Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2732]
Anti-GQ_{1b} Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2662]
Anti-GalNAc-GD_{1a} Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2701]
Anti-Gb_3 Monoclonal Antibody	Mouse IgG2b	0.1mg/vial	[A2506]
Anti-Gb_3 Monoclonal Antibody Biotin Conjugate	Mouse IgG2b	0.1mg/vial	[A2822]
Anti-SGPG (HNK-1) Monoclonal Antibody	Mouse IgG2a	0.1mg/vial	[A2706]
Anti-SSEA-3 Monoclonal Antibody	Mouse IgG3	0.1mg/vial	[A3329]
Anti-SSEA-4 Monoclonal Antibody	Mouse IgG2b	0.1mg/vial	[A3342]

Anti-Sulfated Glycan Antibodies

Sulfated glycans are commonly found in glycosaminoglycans, and their diverse sulfation patterns are responsible for a wide variety of biological interactions. For example, 6-sulfo LacNAc (slan) and sulfated sialyl-Lewis X have been reported to respectively act as P-selectin ligand and L-selectin ligand in humans and mice during cell adhesion mediated by selectins involved lymphocyte homing. Here, TCI offers antibody products useful for detecting these carbohydrate ligands.



6-Sulfo LacNAc



6-Sulfo-sialyl Lewis X

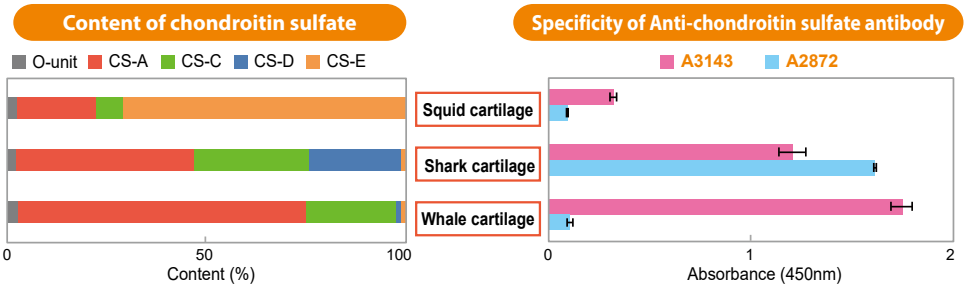
Product Name	Isotype	Size	Product Number
Anti-6-sulfo LacNAc Monoclonal Antibody (AG105)	Mouse IgM	0.1mg/vial	[A3251]
Anti-6,6'-disulfo LacNAc Monoclonal Antibody (L4L4-8)	Mouse IgM	0.1mg/vial	[A3252]
Anti-Sialyl 6,6'-disulfo LacNAc Monoclonal Antibody (G270-16)	Mouse IgM	0.1mg/vial	[A3253]
Anti- Sialyl 6-sulfo Lewis X Monoclonal Antibody (G152)	Mouse IgM	0.1mg/vial	[A3399]
Anti-α2-6-Sialylated 6-sulfo LacNAc Monoclonal Antibody (KN343)	Mouse IgM	0.1mg/vial	[A3428]

Please inquire for pricing and availability of listed products to our local sales representatives.

Anti-Glycosaminoglycan Antibodies

The extracellular matrix (ECM) is an essential element for higher organisms to form cells, tissues, and organs; to control cell-cell connections and functions. The ECM also greatly affects several biological phenomena (such as development, aging, inflammation, wound healing, and immunity). Glycosaminoglycans (GAGs), such as chondroitin sulfate, hyaluronic acid and keratan sulfate, are major components of the ECM and play an important role. Analysis of glycosaminoglycan is very difficult, especially when performing in situ analysis of cells and tissues. Thus, antibodies are particularly important as detection tools.

Anti-chondroitin sulfate antibody can be utilized for detection of the chondroitin sulfate A or D



These chondroitin sulfate were coated on ELISA plate. These antigens and anti-chondroitin sulfate antibodies were reacted at appropriate time, then fist antibodies were detected using appropriate secondary antibodies.

Product Name	Isotype	Size	Product Number
Anti-Chondroitin Sulfate A Monoclonal Antibody (LY111)	Mouse IgM	0.1mg/vial	[A3143]
Anti-Chondroitin Sulfate D Monoclonal Antibody (MO-225)	Mouse IgM	0.1mg/vial	[A2872]
Anti-Keratan Sulfate Monoclonal Antibody (R-10G)	Mouse IgG1	0.1mg/vial	[A2968]
Anti-Perlecan Monoclonal Antibody (HK-102)	Rat IgG2a	0.1mg/vial	[A3342]

Anti-Blood Group Antigen Antibodies

While the ABO blood group, comprising the carbohydrate antigens H, A, and B, is the most well-known of the blood groups, various less prominent blood groups have also been described. Lewis a (Le^a) and Lewis b (Le^b) are carbohydrate antigens of one such group, Lewis blood group. Other carbohydrate antigens structurally related to these antigens include sialyl Lewis a (sLe^a), Lewis x (Le^x), its sialylated derivative, sialyl Lewis x (sLe^x), and Lewis y (Le^y). Lex is the epitope for SSEA1, an undifferentiated marker for mouse embryonic stem cells (ES cells). sLe^x is a ligand for the cell adhesion molecule E-selectin which is involved in the migration of neutrophils to sites of inflammation. Additionally, expression of Le^x, Le^y, sLe^a, and sLe^x is increased in cancers and is involved in cancer progression. Indeed, sLe^a (CA19-9) is used as a tumor marker. We have a wide selection of antibodies against such carbohydrate antigens.

Product Name	Isotype	Size	Product Number
Anti-Lewis X Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2578]
Anti-Lewis Y Monoclonal Antibody	Mouse IgG3	0.1mg/vial	[A2510]
Anti-Sialyl Lewis A Monoclonal Antibody (1H4)	Mouse IgG3	0.1mg/vial	[A2584]
Anti-Sialyl Lewis A Monoclonal Antibody (2D3)	Mouse IgM	0.1mg/vial	[A2509]
Anti-Sialyl Lewis X Monoclonal Antibody	Mouse IgM	0.1mg/vial	[A2849]

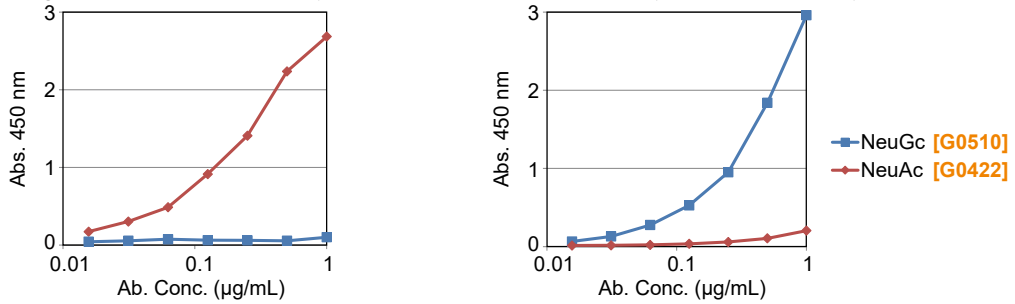
Anti-NeuGc Polyclonal Antibodies

***N*-Acetylneuraminic Acid (NeuAc) and *N*-Glycolylneuraminic Acid (NeuGc) are the two major forms of sialic acid found in mammals. Humans are unable to synthesize Neu5Gc due to a mutation in the gene encoding the enzyme responsible for Neu5Gc synthesis. Humans naturally possess antibodies against Neu5Gc glycan structures, and this is responsible for the immunogenicity of therapeutic proteins containing Neu5Gc glycan epitopes. Therefore, a method for the detection of Neu5Gc is required.**

Anti-NeuGc Polyclonal Antibody	0.05mg/vial **[A3240]**
Anti-NeuGc Polyclonal Antibody Biotin Conjugate	0.05mg/vial **[A3294]**
Anti-NeuGc Polyclonal Antibody FITC Conjugate	0.05mg/vial **[A3295]**
Anti-NeuGc Polyclonal Antibody R-PE Conjugate	0.05mg/vial **[A3360]**
Anti-NeuGc Polyclonal Antibody HRP Conjugate	0.05mg/vial **[A3397]**

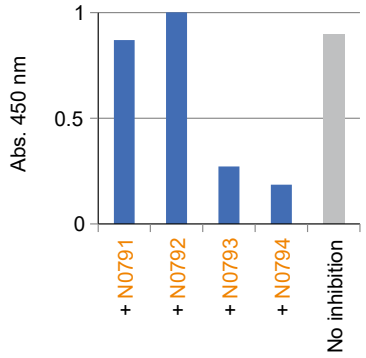
Anti-NeuGc Polyclonal Antibody reacts NeuGc but not NeuAc

Anti-GM_3 Monoclonal Antibody [A2582] **Anti-NeuGc Polyclonal Antibody [A3240]**



The glycolipids coating the ELISA plates reacted with these antibodies. These primary antibodies were then detected using appropriate secondary antibodies.

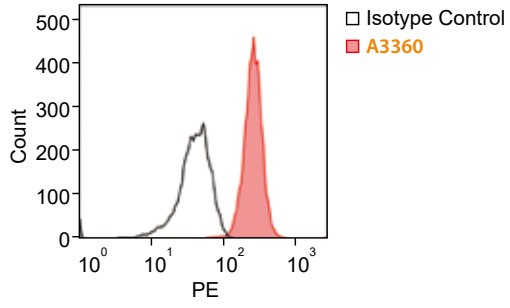
Binding of Anti-NeuGc Antibody is inhibited by NeuGcα(2-3)Gal and NeuGcα(2-6)Gal



ELISA plates were coated with BSM. Anti-NeuGc antibodies and/or inhibitors were incubated in tubes and then made to react with the bound BSM. The primary antibodies were then detected using appropriate secondary antibodies. The inhibitors used are listed below.

Neu5Acα(2-3)Galβ MP Glycoside [N0791]
Neu5Acα(2-6)Galβ MP Glycoside [N0792]
Neu5Gcα(2-3)Galβ MP Glycoside [N0793]
Neu5Gcα(2-6)Galβ MP Glycoside [N0794]

Detection of NeuGc in miniature pig granulocytes by flow cytometry



Granulocytes were collected by hemolyzing the blood of miniature pigs. The granulocytes were incubated (4 °C, 20 minutes) with isotype control (black line) or anti-NeuGc polyclonal antibody R-PE conjugate **[A3360]** (red line) adjusted to 10 μg/mL. Afterward, it was measured using a flow cytometer.

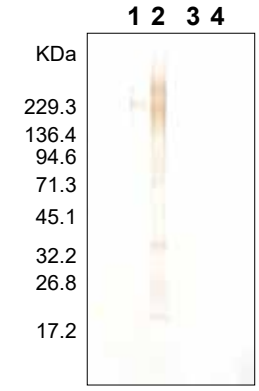
Please inquire for pricing and availability of listed products to our local sales representatives.

Anti-αGal Polyclonal Antibodies

Anti-αGal antibody exists as a natural antibody in humans. Binding of this antibody to αGal antigens (αGal epitope) expressed on porcine xenograft surfaces are a major factor for determining engraft survival. Recently, it has been observed that therapeutic antibodies and cell processing material for reproductive medicine contain the αGal epitope, which indicates the importance of rapid detection of αGal epitope.

Anti-αGal Polyclonal Antibody (Chicken)	0.05mg/vial	[A3123]
Anti-αGal Polyclonal Antibody Biotin Conjugate	0.05mg/vial	[A3144]
Anti-αGal Chicken Polyclonal Antibody HRP Conjugate	0.05mg/vial	[A3195]
Anti-αGal Polyclonal Antibody FITC Conjugate	0.05mg/vial	[A3337]
Anti-αGal Polyclonal Antibody R-PE Conjugate	0.05mg/vial	[A3354]

Anti-αGal antibody can be utilized for detection of the αGal epitope on glycoproteins



Western blotting analysis performed using an anti-αGal polyclonal antibody biotin conjugate [A3144].

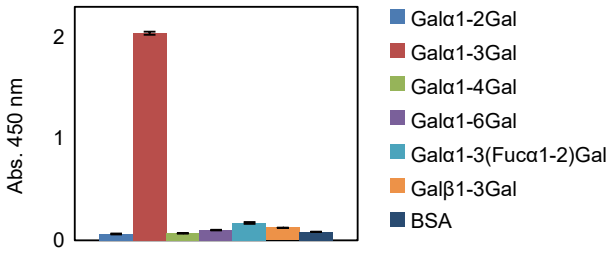
Lane 1: Thyroglobulin, porcine thyroid gland.

Lane 2: Laminin, Engelbreth-Holm-Swarm murine sarcoma basement membrane.

Lane 3: Thyroglobulin treated with α1-3, 4, 6 galactosidase.

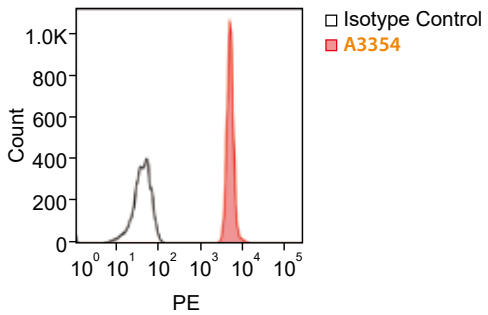
Lane 4: Laminin treated with α1-3, 4, 6 galactosidase.

Anti-αGal polyclonal antibody shows high specificity for αGal epitope



Glycoconjugates coated on ELISA plates. Results following epitope and anti-αGal antibodies incubation. Primary antibodies were detected using appropriate secondary antibodies.

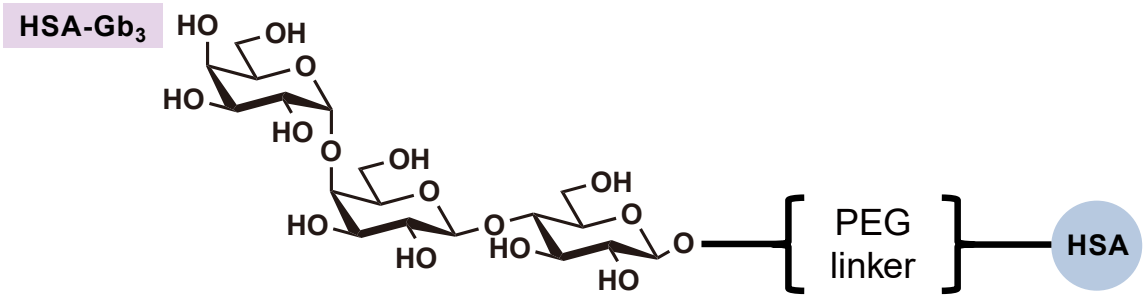
Detection of αGal in miniature pig granulocytes by flow cytometry



Granulocytes were collected by hemolyzing the blood of miniature pigs. The granulocytes were incubated (4 °C, 20 minutes) with isotype control (black line) or anti-αGal polyclonal antibody R-PE conjugate [A3354] (red line) adjusted to 10 µg/mL. Afterward, it was measured using a flow cytometer.

Antigen Sugar-conjugated Proteins

TCI offers carbohydrate-conjugated human serum albumin (HSA) which is manufactured using high-purity synthesized carbohydrates. Several sugar-conjugates are available, and it is also possible to manufacture the sugar-conjugates according to customer specifications. For more details on the products and contracts, please contact us.



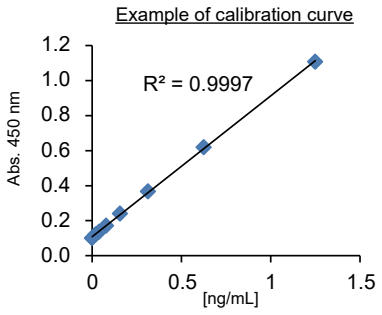
HSA-Gb_3	0.1mg/vial	[H1718]
HSA-Gb_5	0.1mg/vial	[H1777]
HSA-Lewis	0.1mg/vial	[H1719]
HSA-Sialyl Lewis X	0.1mg/vial	[H1730]
HSA-GM_1 Pentasaccharide	0.1mg/vial	[H1767]
HSA-Globo-H	0.1mg/vial	[H1794]
HSA-L1-L1	0.1mg/vial	[H1782]

Anti-Protein A Antibodies

Anti-Protein A Chicken Polyclonal Antibody	0.1mg/vial	[A3044]
Anti-Protein A Chicken Polyclonal Antibody Biotin Conjugate	0.05mg/vial	[A3045]
Anti-Protein A Chicken Polyclonal Antibody HRP Conjugate	0.05mg/vial	[A3187]

High-sensitive detection of Protein A by sandwich-ELISA

1. Dilute anti-Protein A antibody [A3044] with sodium carbonate buffer (pH 8.5), and coat on an ELISA plate.
2. Block with 1% BSA / TBS-T for 2 hours.
3. After washing 3 times with TBS-T, add the sample to each well and incubate for 30 minutes.
4. After washing 3 times with TBS-T, add 1 µg/mL of anti-Protein A antibody biotin conjugate [A3045] to each well and incubate for 30 minutes.
5. After washing 3 times with TBS-T, add SA-HRP [S0972] to each well and incubate for 30 minutes.
6. After washing 3 times with TBS-T, add TMB solution and incubate for 30 minutes.
7. Stop the reaction by adding 1 N HCl, and measure the absorbance at 450nm.



Please inquire for pricing and availability of listed products to our local sales representatives.

Protein A

Protein A is a bacterial cell wall component from *Staphylococcus aureus* that specifically binds to the Fc region of IgG derived from various species including human, rabbit, mouse and cow. Our products consist of a recombinant protein A mutant which allows elution of antibodies under mild conditions (pH 4.0). As there is no change in antibody binding affinity, it can be used in the same way as normal protein A.

Protein A Recombinant, expressed in *Escherichia coli*	5mg/vial [P2366]
Protein A Biotin Conjugate	1mg/vial [P2407]
Protein A HRP Conjugate	0.2mg/vial [P2466]
Protein A Agarose	2mL/vial [P2461]

Purification of human IgG using P2461

Protein A agarose in which protein A is bound to an agarose resin by a covalent coupling method can be used in antibody purification and immunoprecipitation. Antibody purification using protein A agarose usually requires an acidic buffer solution between pH 2.5 and pH 3.0 during elution steps. However, this frequently causes the antibody to undergo acid denaturation, changing its higher-order structure, resulting in antibody aggregation and inactivation. TCI's protein A agarose **[P2461]** uses a genetically modified protein A mutant which allows for the elution of antibodies under mild conditions (pH 4.0), under which most antibodies do not denature, as shown in Figure 1.

Protocol:
1. Fill the column with protein A agarose (Product No. P2461), and equilibrate it with binding buffer.
2. Add human IgG.
3. Wash the resin with binding buffer, and then elute antibodies with pH 4.0 and pH 3.0 elution buffer.

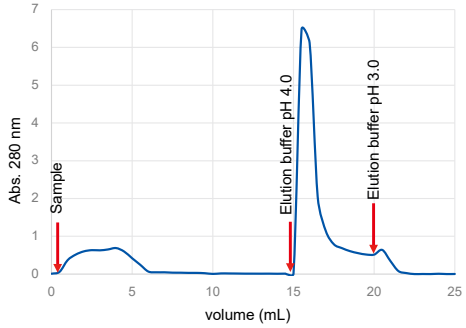


Figure 1. Purification of human IgG using (Product No. P2461)

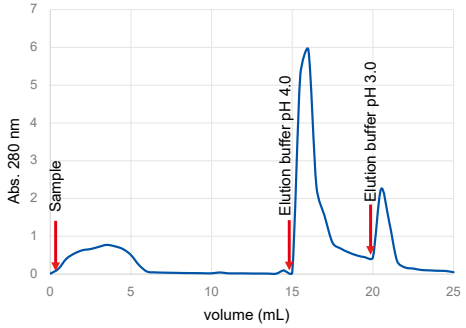


Figure 2. Purification of human IgG using other manufacturer's products

The majority of applied human IgG was successfully eluted at pH4.0 when using P2461.

Protein G

Protein G is a bacterial cell wall component of Group G *Streptococci* strain. It binds specifically to the Fc region of immunoglobulins (especially IgG) and weakly to the Fab fragment.

Protein G Recombinant, expressed in *Escherichia coli*	1mg/vial [P2808]
Protein G Biotin Conjugate	0.2mg/vial [P2959]
Protein G HRP Conjugate	0.2mg/vial [P2962]

Protein L

Protein L is a cell wall molecule from the bacterial species *Peptostreptococcus* magnus. It binds immunoglobulin light chains in a wide range of species including human, mouse, rat, pig, and hamster, and can bind to any immunoglobulin isoform containing a κ light chain (IgG, IgM, IgA, IgE, and IgD). It can also bind single-chain antibodies (scFv) and Fab fragments with κ light chains.

Protein L Recombinant, expressed in *Escherichia coli* 1mg/vial [P3059]

Protein L Biotin Conjugate 0.2mg/vial [P2998]

Protein L HRP Conjugate 0.2mg/vial [P2999]

Anti-Endo-M Antibodies

Anti-Endo-M Polyclonal Antibody 0.2mg/vial [A2958]
Immunogen : *endo*-β-*N*-Acetylglucosaminidase (Endo-M) Isotype : Rabbit IgG

Anti-Endo-M Polyclonal Antibody Biotin Conjugate 0.1mg/vial [A2959]

Related Products: Enzymes which Transfers the Intact Oligosaccharides

***endo*-β-*N*-Acetylglucosaminidase (=Endo-M)** 100mUnits/vial [A1651]
Recombinant: from *Mucor hiemalis* expressed in *Candida boidinii*

Glycosynthase (Endo-M-N175Q) 100mUnits/vial [G0365]
Recombinant: from *Mucor hiemalis* expressed in *Escherichia coli*

Endo-M-W251N 500mUnits/vial [E1339]
Recombinant: from *Mucor hiemalis* expressed in *Escherichia coli*

Anti-Influenza Virus Antibodies

Anti-Influenza A Virus Neuraminidase N1 Monoclonal Antibody
Immunogen : Influenza A/Brijing/262/95 Clone name : 2-3B Isotype : Mouse IgG1 0.2mL [A2407]

Anti-Influenza A Virus Hemagglutinin H3 Monoclonal Antibody
Immunogen : Influenza A/Sydney/5/97 Clone name : 1G8 Isotype : Mouse IgG3 0.2mL [I0779]

Anti-Influenza A Virus Neuraminidase N2 Monoclonal Antibody
Immunogen : Influenza A/Sydney/5/97 Clone name : 1-4B Isotype : Mouse IgG1 0.2mL [A2380]

Anti-Influenza A Virus Nucleoprotein Monoclonal Antibody
Immunogen : Influenza A/Beijing/262/95 Clone name : 17 Isotype : Mouse IgG2a 0.2mL [A2406]

Please inquire for pricing and availability of listed products to our local sales representatives.

Anti-Tag Antibodies

Anti-DYKDDDDK Antibody

Mouse Anti-DYKDDDDK Monoclonal Antibody 0.1mg/vial [M3389]
Mouse Anti-DYKDDDDK Monoclonal Antibody Biotin Conjugate 0.05mg/vial [M3400]
Mouse Anti-DYKDDDDK Monoclonal Antibody HRP Conjugate 0.05mg/vial [M3712]

Anti-HHHHHH (6xHis) Antibody

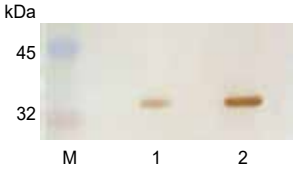
Anti-6xHis Monoclonal Antibody (6A12) 0.1mg/vial [A2957]
Immunogen : HHHHHH (6xHis) Isotype : Mouse IgG1
Anti-6xHis Monoclonal Antibody (6A12) Biotin Conjugate 0.05mg/vial [A3010]
Anti-6xHis Monoclonal Antibody (6A12) HRP Conjugate 0.05mg/vial [A3075]

Anti-Glutathione *S*-Transferase (GST) Antibody

Anti-GST Monoclonal Antibody 0.1mg/vial [A3175]
Immunogen : Glutathione *S*-transferase (GST) Isotype : Mouse IgG2a
Anti-GST Monoclonal Antibody Biotin Conjugate 0.05mg/vial [A3226]

GST Detection by A3175

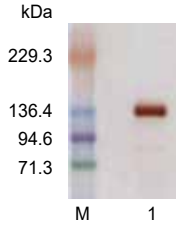
Western blotting
(GST : 0.1 µg/lane)



Lane M : Molecular weight marker
Lane 1 : GST (nonreduction)
Lane 2 : GST (reduction)

DYKDDDDK Tag Detection by M3400

Western blotting
(human ITGAX fused with DYKDDDDK tag : 0.1 mg/lane)



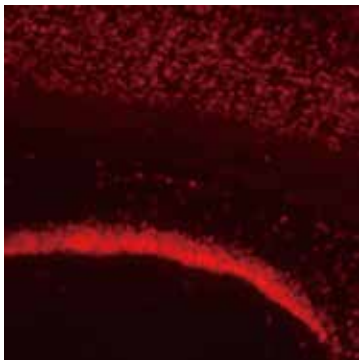
Lane M : Molecular weight marker
Lane 1 : human ITGAX fused with DYKDDDDK tag (reduction)

Anti-Cell Marker Antibodies

Mouse Anti-NeuN Monoclonal Antibody 0.1mg/vial [M3586]
Mouse Anti-NeuN Monoclonal Antibody HRP Conjugate 0.05mg/vial [M3755]

NeuN (RNA binding protein fox-1 homolog 3) is a nuclear protein mainly expressed in postmitotic neurons. Anti-NeuN antibodies are useful markers of mature neurons and widely used in embryology and neuroscience.

Immunofluorescence of Mouse Tissue Section Stained Using M3586



Primary antibody :
Mouse Anti-NeuN Monoclonal Antibody [M3586]
Secondary antibody :
Goat Anti-Mouse IgG_1 Fab Fragment Cyanine 3 Conjugate [G0598]

4 µg of M3586 and 3 µg of G0598 were mixed and incubated for 1.5 hours at 37°C. The mixture was diluted 500 times, added to a mouse brain section, and incubated overnight at room temperature with shaking. After washing, sections were observed via a fluorescence microscope.

Secondary Antibodies and Other Antibodies

Anti-Mouse IgG

Goat Anti-Mouse IgG	1mg/vial	[G0386]
Goat Anti-Mouse IgG Biotin Conjugate	0.1mg/vial	[G0387]
Goat Anti-Mouse IgG HRP Conjugate	0.1mg/vial	[G0407]
Goat Anti-Mouse IgG FITC Conjugate	0.1mg/vial	[G0406]
Goat Anti-Mouse IgG R-PE Conjugate	0.1mg/vial	[G0569]
Goat Anti-Mouse IgG_1 Fab Fragment Cyanine 3 Conjugate	0.05mg/vial	[G0598]

Anti-Mouse IgM

Goat Anti-Mouse IgM	1mg/vial	[G0408]
Goat Anti-Mouse IgM Biotin Conjugate	0.1mg/vial	[G0432]
Goat Anti-Mouse IgM HRP Conjugate	0.1mg/vial	[G0417]
Goat Anti-Mouse IgM FITC Conjugate	0.1mg/vial	[G0453]

Anti-Rabbit IgG

Goat Anti-Rabbit IgG	1mg/vial	[G0388]
Goat Anti-Rabbit IgG Biotin Conjugate *	0.1mg/vial	[G0597]
Goat Anti-Rabbit IgG HRP Conjugate	0.1mg/vial	[G0418]
Goat Anti-Rabbit IgG FITC Conjugate	0.1mg/vial	[G0452]
Goat Anti-Rabbit IgG R-PE Conjugate	0.1mg/vial	[G0577]

Anti-Chicken IgY

Sheep Anti-Chicken IgY	1mg/vial	[S0998]
Sheep Anti-Chicken IgY Biotin Conjugate	0.1mg/vial	[H1619]
Sheep Anti-Chicken IgY HRP Conjugate	0.1mg/vial	[S0999]

Anti-HRP Antibody

Anti-HRP Rabbit Polyclonal Antibody 0.2mL [A2250]

Immunogen : Horseradish Peroxidase Isotype: Rabbit IgG

Anti-Human IgG

Anti-Human IgG Fc *C*-terminus Monoclonal Antibody 0.1mg/vial [A3277]

Immunogen : Synthetic peptide corresponding to human IgG Fc C terminus Isotype: MouseIgG1

Mouse Anti-Human IgG Fc	0.1mg/vial	[M2977]
Mouse Anti-Human IgG Fc Biotin Conjugate	0.1mg/vial	[M3053]

*G0597 is the successor to Anti-Rabbit IgG Biotin Conjugate (Product Number: G0389).
Please use G0597 alternatively if you have used G0389.

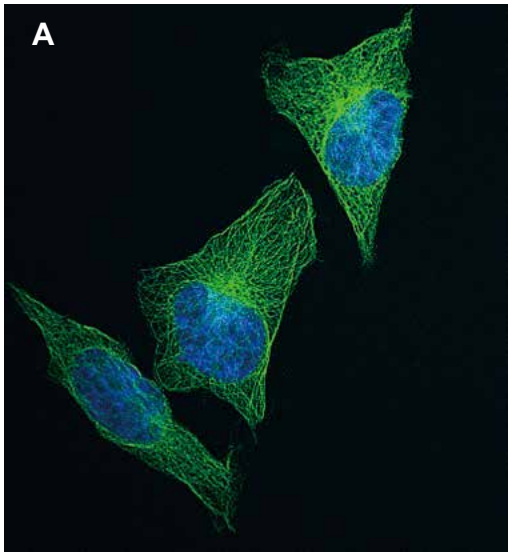
Streptavidins

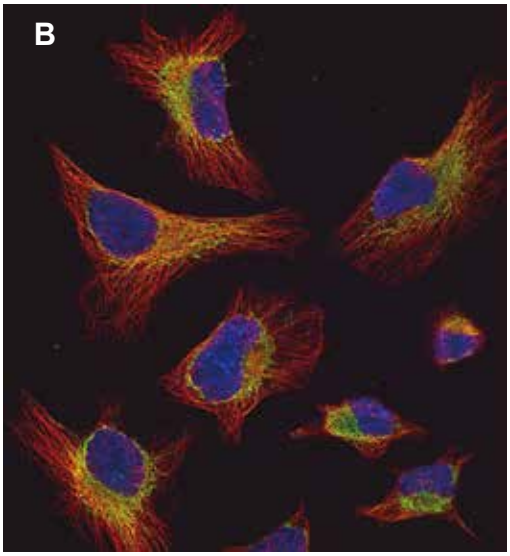
Streptavidin from *Streptomyces avidinii*	1mg/vial	[S0951]
Streptavidin HRP Conjugate	0.1mg/vial	[S0972]
Streptavidin FITC Conjugate	0.1mg/vial	[S0966]
Streptavidin DTBTA-Eu^{3+} Conjugate	0.1mg/vial	[S0993]
Streptavidin R-PE Conjugate	0.1mg/vial	[T3885]
Streptavidin Maleimide Conjugate	0.5mg/vial	[T3531]

Please inquire for pricing and availability of listed products to our local sales representatives.

Fluorescent Labeled Secondary Antibodies and Fluorescent Cell Stains

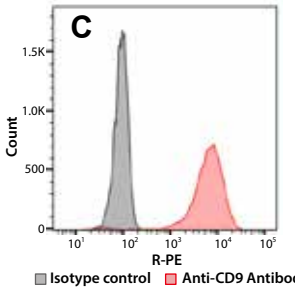
Applications





(A) The HeLa cells were incubated with properly diluted primary antibody (Mouse Anti α-Tubulin IgG) and were further incubated with Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin FITC Conjugate [S0966] (green fluorescence). And then the nuclei was stained with DAPI 2HCl [A2412] (blue fluorescence). (Laser Scanning Microscope: Olympus FLUOVIEW FV 3000)

(B) The nuclei of HeLa cells was stained with Bisbenzimide H 33258 [H1343] (blue fluorescence). α-Tubulin was stained with anti-α-tubulin antibody and Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin R-PE Conjugate [T3885] (red fluorescence). Mitochondria was stained with primary antibody and Goat Anti-Rabbit IgG FITC Conjugate [G0452] (green fluorescence)**. (Laser Scanning Microscope: Olympus FLUOVIEW FV 3000)



(C) The HeLa cells were incubated with Mouse Anti-CD9 Antibody (red line) or Mouse IgG2aκ isotype control (black line). Subsequently, both were stained with Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin R-PE Conjugate [T3885]. (Flow cytometer: Sysmex RF-500)

**Please refer to our product page for staining procedure.
R-PE/FITC-labeled anti-Mouse IgG or anti-Rabbit IgG antibodies and streptavidins can be used for fluorescence immunostaining and flow cytometry.

Goat Anti-Mouse IgG FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[G0406]
Goat Anti-Mouse IgM FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[G0453]
Goat Anti-Rabbit IgG FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[G0452]
Streptavidin FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[S0966]
Goat Anti-Mouse IgG R-PE Conjugate	(Red Fluorescence)	0.1mg/vial	[G0569]
Goat Anti-Mouse IgG_1 Fab Fragment Cyanine 3 Conjugate	(Red Fluorescence)	0.05mg/vial	[G0598]
Goat Anti-Rabbit IgG R-PE Conjugate	(Red Fluorescence)	0.1mg/vial	[G0577]
Streptavidin R-PE Conjugate	(Red Fluorescence)	0.1mg/vial	[T3885]
Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate	(Red Fluorescence)	0.1mg/vial	[G0505]
Goat Anti-Rabbit IgG DTBTA-Eu^{3+} Conjugate	(Red Fluorescence)	0.1mg/vial	[G0506]
Streptavidin DTBTA-Eu^{3+} Conjugate	(Red Fluorescence)	0.1mg/vial	[S0993]
DAPI 2HCl	(Blue Fluorescence)	5mg	[A2412]
DAPI 2HCl (1mg/mL in Water)	(Blue Fluorescence)	0.2mL x 5vial	[D5888]
Bisbenzimide H 33258	(Blue Fluorescence)	25mg	[H1343]
Bisbenzimide H 33258 (1mg/mL in Water)	(Blue Fluorescence)	0.2mL x 5vial	[B6236]

*Some products are unavilable in the Americas and China.
*The high-sensitivity detection of DTBTA-Eu^{3+} labeled probes requires time-resolved fluorometry.

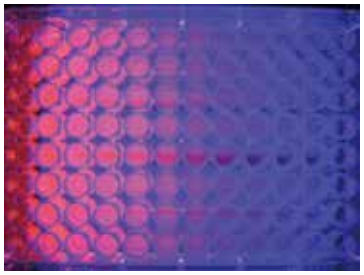
Related Product **Fluoro-Long Antifade Mounting Medium** 10mg [A2083]

Europium Fluorophore DTBTA-Eu^{3+}-labeled Proteins

Highly-sensitive Detection Probes for Time-resolved Fluorometry

Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate 0.1mg/vial [G0505]
Goat Anti-Rabbit IgG DTBTA-Eu^{3+} Conjugate 0.1mg/vial [G0506]
Streptavidin DTBTA-Eu^{3+} Conjugate 0.1mg/vial [S0993]

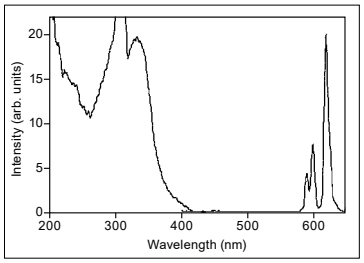
Advantages



No cross talk of excitation light

- Excitation wavelength Ex_{max} : 335 nm
- Emission wavelength Em_{max} : 616 nm

Sharpened emission spectrum
Large Stokes shift (the difference in wavelength between positions of the band maxima of the absorption and emission spectra)



Stable fluorescence in various aqueous buffers

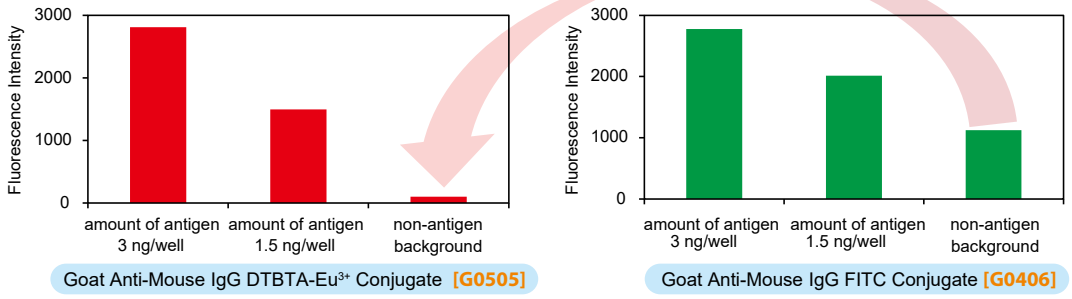
Available in Tris, TE, PBS, etc., for wide use

Long fluorescent life time (τ = 1.02 ms)

Time-resolved fluorometric measurement can remove background fluorescence from the sample matrix and often gives detectability better than one order of magnitude compared to those of conventional fluorometric assays.

Comparison of secondary antibody conjugated to DTBTA-Eu^{3+} or FITC

Time-resolved fluorometric measurement can remove background fluorescence!
To obtain a high SN ratio



Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate [G0505]
Goat Anti-Mouse IgG FITC Conjugate [G0406]

<Assay condition>
Dilute the Mouse IgG to each concentration. Coat 96-well plates with diluted Mouse IgG. Block the plates with BSA/TBST. Incubate with Goat Anti-Mouse IgG Conjugates labeled by DTBTA-Eu^{3+} or FITC at 2.5 µg/mL. After incubation, measure the fluorescence intensity on a plate reader.
DTBTA-Eu^{3+}; excitation=340 nm, emission=620 nm. Lag Time : 450 µsec
FITC; excitation=485 nm, emission=520 nm.

Related Products

Anti-DTBTA-Eu^{3+} Antibody

Anti-DTBTA-Eu^{3+} Rabbit Polyclonal Antibody 0.5mL [A2239]
Anti-DTBTA-Eu^{3+} Rabbit Antiserum 0.5mL [A2181]

DTBTA-Eu^{3+} Labeling Reagent

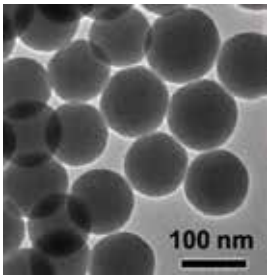
ATBTA-Eu^{3+} 10mg [A2083]

Please inquire for pricing and availability of listed products to our local sales representatives.

Fluorescent Organosilica Particles

Organosilica FITC (100nm Diam.) 2mg [O0561]
Organosilica Rhodamine B (100nm Diam.) 2mg [O0573]

Fluorescent image of O0561

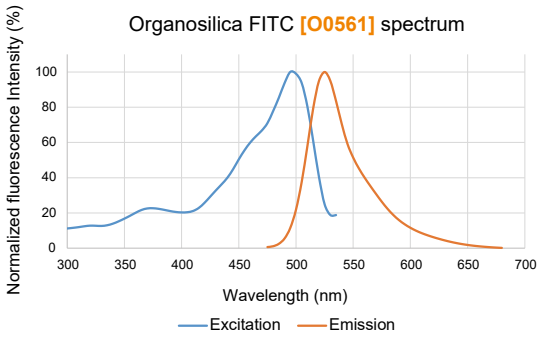


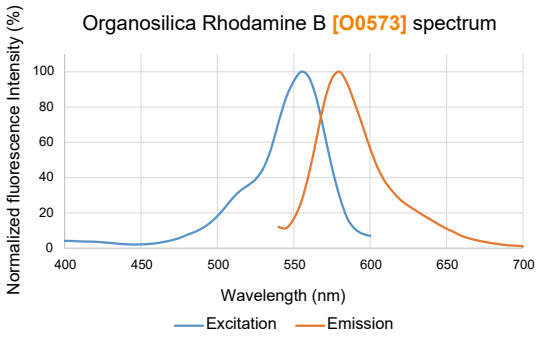
SEM image

Advantages

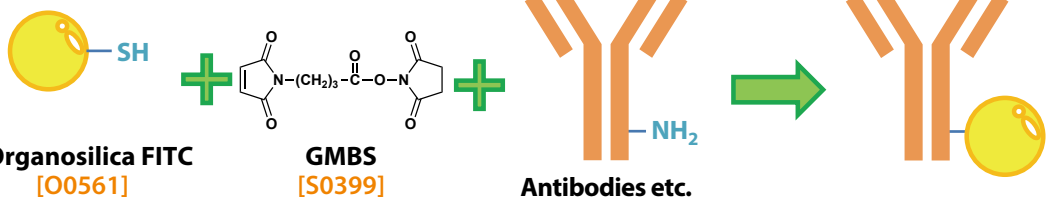
- Wavelength : Ex_{max} 492 nm, Em_{max} 523 nm (O0561)
  Ex_{max} 556 nm, Em_{max} 579 nm (O0573)
- Surface Functionalization : Thiol group (-SH)
- Superior in fluorescence intensity to the conventional FITC or rhodamine B.
- The diameter of these products are 100 nm and these products are suitable for the detection of biomolecules.

Fluorescence spectra





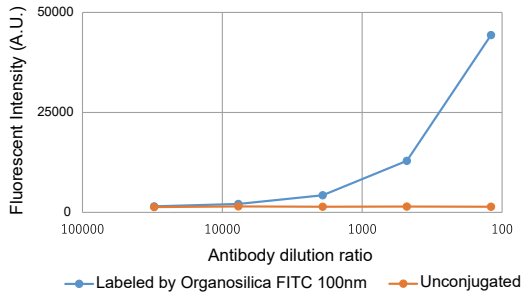
Application



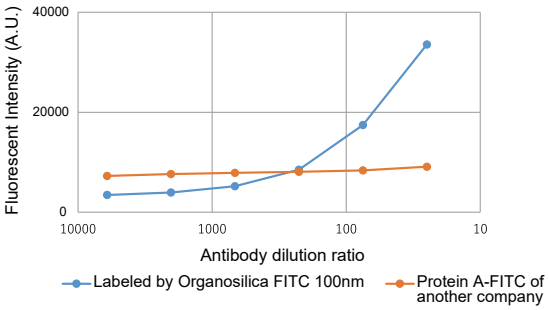
Organosilica FITC [O0561] **GMBS** [S0399] **Antibodies etc.**

Organosilica FITC [O0561] was labeled to various antibodies etc. by the above method. The fluorescence intensity of them at Ex: 485 nm, Em: 520 nm was measured.

Labeling with Goat anti Mouse IgG antibody



Labeling with Protein A



Organosilica FITC 100nm [O0561] could be labeled to various antibodies etc., and they were detected by fluorescence.

These products are commercialized under the instruction of Prof. Michihiro Nakamura.

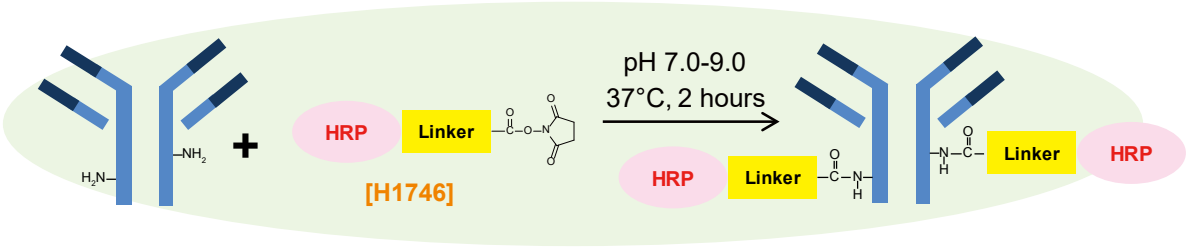
Peroxidase (HRP) Labeling Reagents

Horseradish Peroxidase Maleimide Conjugate (0.5mg×3) 1set [H1621]
Horseradish Peroxidase NHS Ester Conjugate (0.2mg×3) 1set [H1746]

Advantages

- **H1746 contains an *N*-hydroxysuccinimidyl ester (NHS) moiety and can be used to readily label proteins and peptides that have an amino group (-NH_2).**
- **H1621 can be used for the conjugation to free thiol-containing proteins and peptides due to its thiol-reactive maleimide group.**
- **Each protein conjugate is packaged for single use purposes and thus does not require weighing prior to use.**

Application : HRP-labelling of an antibody with H1746



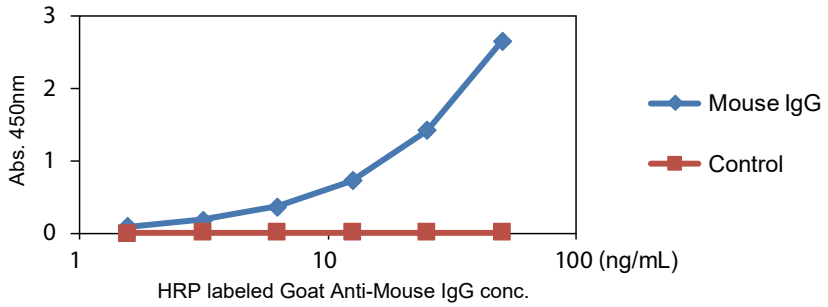
Here is an example of HRP labeling of an antibody (Goat Anti-Mouse IgG) conjugated with H1746.
For more information, see the product detail page of H1746 on TCI website.

Protocol

1. Dissolve the target antibody at 10 mg/mL in 0.1 M Sodium Bicarbonate buffer (pH 8.5).*
2. Add the antibody solution into H1746 vial, and mix well.
3. Incubate for 2 hours at 37 °C.
4. To quench the reaction, add 200 µL of 100mM Tris-HCl buffer pH 7.5.
5. Incubate for 1 hour at 37 °C.

*Tris buffer and other amine containing buffers also interfere with the labeling reaction. It is recommended to use the amine free buffer (e.g. PBS, Phosphate buffer, Borate buffer, Bicarbonate buffer) pH range 7-9.

Activity of HRP labeled antibody

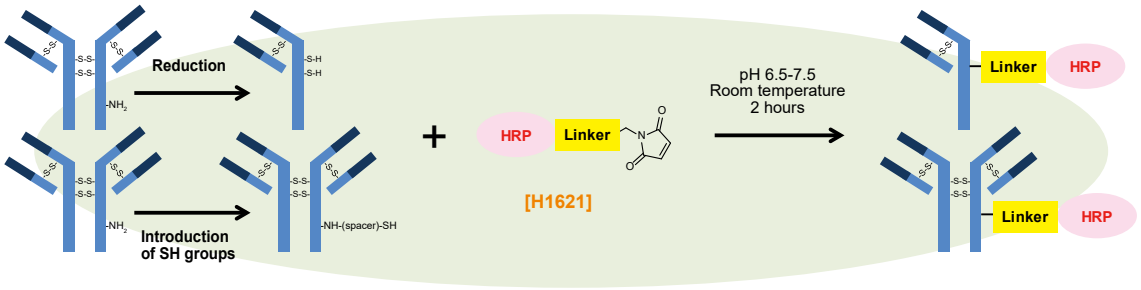


HRP labeled Goat Anti-Mouse IgG conc.

Goat Anti-Mouse IgG labeled with the HRP using H1746 was tested by ELISA for detection of a Mouse IgG coated on a plate. Mouse IgG could be detected sufficiently even if the labeled antibody was diluted to 15 ng/mL or more.

Application : HRP-labelling of an antibody with H1621

In case of antibodies without free thiol (SH, sulfhydryl) groups, disulfide moieties in proteins can be reduced by a reductant such as DTT [D3647] or 2-MEA [A0296] to reveal free thiols.
Furthermore, thiol group can be introduced to primary amines by adding SATA [S0431], SATP [S0859] or Traut's reagent [I0820].

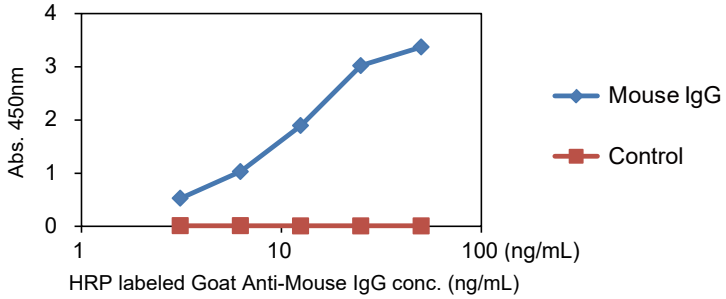


Example protocol for antibody conjugation starts from a reduction of native disulfide bonds in the Goat Anti-Mouse IgG, followed by labeling with the HRP using H1621. For more information, see the product detail page of H1621 on TCI website.

Protocol

1. Add DTT to a final concentration equal to 3 mole equivalents per mole equivalent of antibody present.
2. Incubate for 90 minutes at 37 °C.
3. Purify the reduced IgG by gel filtration or ultrafiltration, dialysis.
4. Add equal amount of H1621 (by weight) to a purified antibody and incubate for 2 hours at room temperature (25 °C).

Activity of HRP labeled antibody



Goat Anti-Mouse IgG labeled with the HRP using H1621 was tested by ELISA for detection of a Mouse IgG coated on a plate. Mouse IgG could be detected sufficiently even if the labeled antibody was diluted to 5 ng/mL or more.

Related Products

Reducing agents for protein disulfide

DTT (= DL-Dithiothreitol)	1g / 5g [D3647]
2-MEA (= 2-Aminoethanethiol Hydrochloride)	25g / 100g / 500g [A0296]
2-Mercaptoethanol	5g / 25g [M1948]
Tris(2-carboxyethyl)phosphine Hydrochloride	1g / 5g / 25g [T1656]

Reagents for introduction of thiol group

SATA (= *N*-Succinimidyl *S*-Acetylthioglycolate)	1g / 5g [S0431]
SATP (= *N*-Succinimidyl 3-(Acetylthio)propionate)	100mg [S0859]
2-Iminothiolane Hydrochloride (= Traut's Reagent)	100mg [I0820]

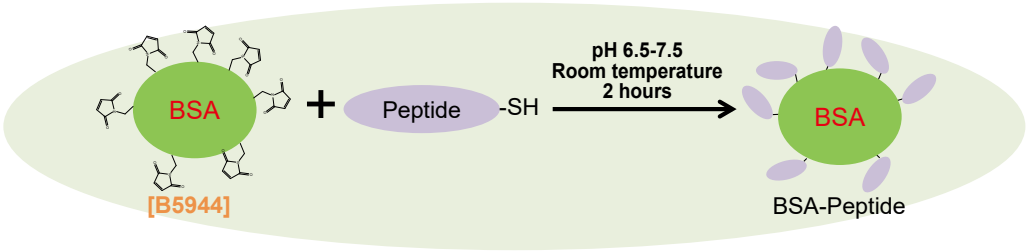
Protein-maleimide Conjugates for Thiol-maleimide Crosslinking

Bovine Serum Albumin Maleimide Conjugate (1mg×3) 1set [B5944]
Horseradish Peroxidase Maleimide Conjugate (0.5mg×3) 1set [H1621]
Streptavidin Maleimide Conjugate (0.5mg×1) 1vial [T3531]

Advantages

- Each product containing a thiol-reactive maleimide group can be used for the conjugation to proteins and peptides containing free thiols.
- Each protein conjugate is packaged for single use purposes and thus does not require weighing prior to use.

Application : Preparation of BSA-Peptide using B5944

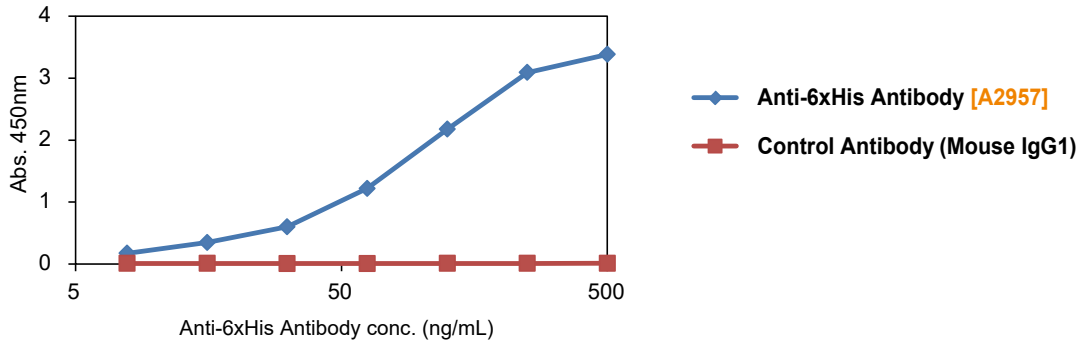


BSA is conjugated to haptens and typically used as an antigen carrier for anti-hapten antibody.
Here we show how to conjugate 6xHis-Cys peptide to B5944.
For more information, see the product detail page of B5944 on TCI website.

Protocol

1. Dissolve the 6xHis-Cys peptide in 0.1 M sodium phosphate, 0.15 M NaCl, 0.1 M EDTA at pH 7.2.
2. Reconstitute B5944 with 100 µL of water.
3. Add 1 mg of 6xHis-Cys peptide to 1 mg of B5944 and Incubate for 2 hours at room temperature (25 °C).

ELISA using the prepared BSA-6His as an antigen



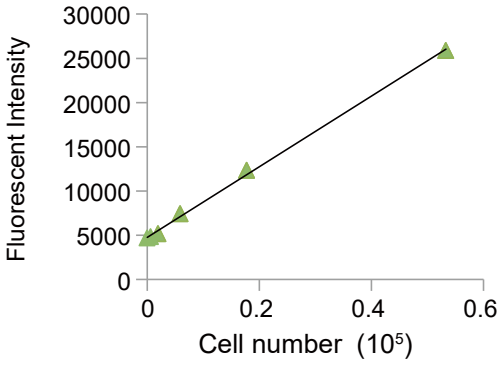
Anti-6xHis Antibody [A2957] was analysed by ELISA using a 0.1 µg/well of BSA-6His coated plate.
Goat Anti-Mouse IgG HRP Conjugate [G0407] was used as the secondary antibody.

Cell Proliferation Assay Reagents

Resazurin (Ready-to-use solution) [for Cell proliferation assay] 25mL [R0195]

Resazurin can be used quantitatively determine cell proliferation, viability, and cytotoxicity. Resazurin, when added to viable cells, is reduced by the cellular enzymatic or chemical reactions converting blue/non-fluorescent resazurin to highly fluorescent resorufin. The assay is simple to perform since the indicator is water-soluble and has low toxicity, thus eliminating the washing/fixing and extraction steps required in other commonly used cell proliferation assays.

Cell viability assay



Application

1. Add **R0195** at a volume equal to 10% of the cell culture media volume.
2. Return cells to the incubator and continue the incubation for 2 - 24 hours.*
3. Measure the fluorescent intensity using 540 - 570 nm excitation and 590 nm emission wavelengths. Absorbance can be measured using a spectro-photometer set at 570 nm.

Resazurin may be added at any time point during the culture period. For measurement of cell proliferation, it is best to add resazurin during the log phase of growth.

Live/Dead Cell Staining Kit [for Cell Staining] 1kit [L0465]

This product is a combination of Calcein-AM, a fluorescent dye for staining live cells, and PI (Propidium Iodide), a fluorescent dye for staining dead cells, allowing simultaneous staining of live and dead cells in approximately 15 minutes. It can be used to observe cells under a fluorescence microscope.

Application

1. Bring Calcein-AM solution and PI solution to room temperature, and add 1 µL Calcein-AM solution and 3 µL PI solution to 1 mL PBS.
2. Collect cells and centrifuge the cell suspension. Remove the supernatant and add PBS to wash the cells. Repeat this washing process twice.
3. Incubate at 37 °C for 15 minutes.
4. Place the stained cell solution on a slide, gently cover with a coverslip, and examine under a microscope.

K562 cells treated with 0-20% DMSO were stained.

As the DMSO concentration increases, the number of live cells (green fluorescence) decreases and the number of dead cells (red fluorescence) increases.

Related Products

MTT [for Biochemical Research]	200mg / 1g [M3297]
ATP-Luciferase Cell Viability Assay Solution (1.0mL×10)	1set [A3495]
MTT Solution [for Cell proliferation assay] (1mL×5)	1set [M3353]
WST-8 Reagent [for Cell Proliferation Assay]	1mL [W0023]
ATP-Luciferase Cell Viability Assay Solution	10mL [A3519]
Intracellular Reactive Oxygen Species (ROS) Detection Assay Kit	1kit [I1265]
Malondialdehyde Measurement Kit	1kit [M3637]

Extraction Buffers for Cells

RIPA Buffer (Ready-to-use) [for Protein extraction]

100mL [R0246]

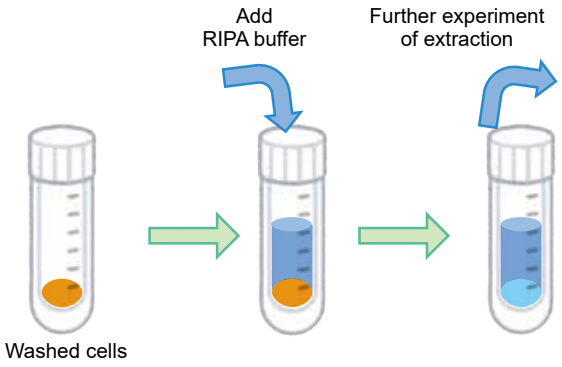
This product is supplied as a ready-to-use solution for the lysis of the cultured mammalian cells. Proteins can be extracted by adding this buffer [R0246] to the cells and the extract can be used directly for further analysis such as western blotting. This product does not include protease inhibitors. Please add a protease inhibitor cocktail, if necessary.

Application

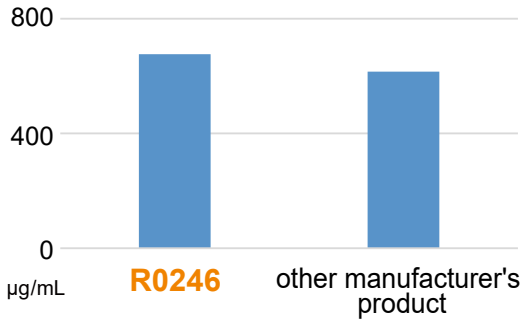
Add the following protease inhibitors to RIPA buffer [R0246].

Leupeptin	10 µg/mL
Pepstatin A	1 µg/mL
Aprotinin	3 µg/mL
AEBSF	1 mM

1. Wash the cultured mouse myeloma-derived cell sp2/0 twice with PBS.
2. Remove PBS and add 200 µL of either cold RIPA buffer [R0246] containing protease inhibitors or the other manufacturer's RIPA buffer containing the same protease inhibitors to 1.0 x 10^6 cells.
3. Incubate the cells for 15 minutes on ice.
4. Centrifuge the cells at 10,000 x g for 10 minutes at 4 °C.
5. Measure the protein concentration of the supernatants.
6. Analyze the supernatants using western blotting.



Extracted Protein Concentration



Western Blotting

The extracts were transferred to a PVDF membrane after electrophoresis.
Anti-β actin antibody was used for detection.
Equal or better detection was observed than that of the other manufacturer's product.

E.coli / Yeast Protein Extraction Buffer

100mL [Y0021]

Y0021 is a ready-to-use solution for protein extraction from cultured *Escherichia coli* (*E. coli*) / yeast cells. By suspending cells in Y0021 and then centrifuging, the supernatant containing proteins can be obtained. Extracted protein can be used in downstream applications such as electrophoresis and western blotting.

Nervous Tissue Protein Extraction Buffer

100mL [B6279]

B6279 is a ready-to-use solution for protein extraction from nervous tissue. By suspending tissue in B6279 and then centrifuging, the supernatant containing proteins can be obtained. Extracted protein can be used in downstream applications such as electrophoresis and western blotting

Peroxidase Substrates

TMB [for ELISA] (Ready-to-use solution)
(= 3,3',5,5'-Tetramethylbenzidine (Ready-to-use solution))

100mL [T3854]

Application

1. Add 100µL of TMB solution **[T3854]** to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. Add 100µL of 1N HCl solution **[H1202]** to each well to terminate the reaction.
4. Measure the absorbance of each well at 450 nm.

When **T3854** reacts with horseradish peroxidase (HRP), a blue colored soluble reaction product appears thus it can be used for ELISA.
This product cannot be used for Western blotting which needs a precipitate.

Figure.
An example of use by the above method

TMB [for Western blotting] (Ready-to-use solution)
(= 3,3',5,5'-Tetramethylbenzidine (Ready-to-use solution))

100mL [T3855]

Application

1. Incubate a blotting membrane with an HRP-conjugated antibody and then wash the membrane.
2. Incubate the washed membrane with TMB solution **[T3855]** until color development.
3. Add deionized water to stop color development.

When **T3855** reacts with HRP, a blue-purple precipitate appears thus it can be used for Western blotting.
This product cannot be used for ELISA which needs a soluble reaction product.

Figure.
An example of Western blotting by the above method

M : molecular weight marker
1 : Target protein A

4-Chloro-1-naphthol (Ready-to-use solution) [for Western blotting]

(= 4-CN (Ready-to-use solution)) 100mL [C3384]

Application

1. Incubate a blotting membrane with an HRP-conjugated antibody and then wash the membrane.
2. Incubate the washed membrane with 4-CN solution [C3384] until color development.
3. Add deionized water to stop color development.

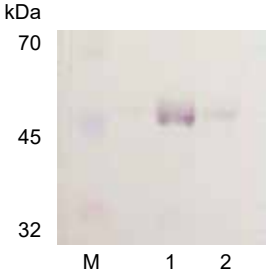


Figure.
An example of Western blotting by the above method

M : molecular weight marker
1 : Target protein B (Middle concentration)
2 : Target protein B (Low concentration)

AzBTS (Ready-to-use solution) [for ELISA]

(= 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid Ammonium Salt) (Ready-to-use solution))

100mL [A3176]

Application

1. Add 100µL of AzBTS solution [A3176] to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. Within 1 hour from start the reaction, measure the absorbance of each well at 405 nm.

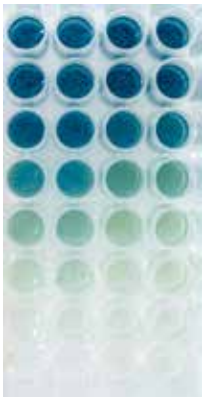


Figure.
An example of use by the above method

Related Products

Sodium Hydroxide (1mol/L in Water)	500mL [S0542]
Hydrochloric Acid (1mol/L)	500mL [H1202]
Peroxidase from Horseradish	100mg / 1g [P0073]
Horseradish Peroxidase Maleimide Conjugate (0.5mg×3)	1set [H1621]
Horseradish Peroxidase NHS Ester Conjugate (0.2mg×3)	1set [H1746]
Anti-6xHis Monoclonal Antibody (6A12) HRP Conjugate	0.05mg/1vial [A3075]
Anti-Protein A Chicken Polyclonal Antibody HRP Conjugate	0.05mg/1vial [A3187]
Anti-αGal Chicken Polyclonal Antibody HRP Conjugate	0.05mg/1vial [A3195]
Anti-NeuGc Polyclonal Antibody HRP Conjugate	0.05mg/1vial [A3397]
Goat Anti-Mouse IgG HRP Conjugate	0.1mg/1vial [G0407]
Goat Anti-Mouse IgM HRP Conjugate	0.1mg/1vial [G0417]
Goat Anti-Rabbit IgG HRP Conjugate	0.1mg/1vial [G0418]
Sheep Anti-Chicken IgY HRP Conjugate	0.1mg/1vial [S0999]
Protein A HRP Conjugate	0.2mg/1vial [P2466]
Streptavidin HRP Conjugate	0.1mg/1vial [S0972]

Please inquire for pricing and availability of listed products to our local sales representatives.

Soluble Substrates (for ELISA etc.)

For such as ELISA, substrates generating soluble dyes with peroxidase.

AzBTS (= 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid Ammonium Salt))	1g	[A2166]
OPD·2HCl (= 1,2-Phenylenediamine Dihydrochloride)	1g	[P1144]
OPD (= 1,2-Phenylenediamine)	1g / 5g	[P1805]
TMB (= 3,3',5,5'-Tetramethylbenzidine)	1g / 5g	[T2573]

Soluble Substrates (for determining H_2O_2)

Substrates generating soluble dyes for determining hydrogen peroxidase (H_2O_2) by various enzyme reactions.

4-AA·2HCl (= 4-Aminoantipyrine Hydrochloride)	5g / 25g	[A0257]
4-AA (= 4-Aminoantipyrine)	1g / 5g	[A2254]
5-ASA (= 5-Aminosalicylic Acid) *1	5g / 25g	[A2291]
DCHBS (= 3,5-Dichloro-2-hydroxybenzenesulfonic Acid Sodium Salt) *1	25g	[D1928]
2,4-DCP (= 2,4-Dichlorophenol) *1	1g / 5g	[D3865]
DMA (= *N*,*N*-Dimethylaniline) *1	1g / 5g	[D3866]
DMT (= *N*,*N*-Diethyl-*m*-toluidine) *1	1g / 5g	[D3868]
TOOS (= Sodium 3-[Ethyl(*m*-tolyl)amino]-2-hydroxy-1-propanesulfonate) *1	1g / 5g	[S0805]
ALPS (= Sodium 3-(*N*-Ethylanilino)propanesulfonate) *1	200mg / 1g	[S0817]
ADOS (= Sodium 3-(*N*-Ethyl-3-methoxyanilino)-2-hydroxy-1-propanesulfonate) *1	200mg / 1g	[S0826]
HDAOS (= *N*-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline Sodium Salte) *1	200mg	[S0827]
MBTH·HCl (= 3-Methyl-2-benzothiazolinonehydrazone Hydrochloride)	1g / 5g	[M2155]

*1 : Used together with A2254 (or A0257)

Precipitate Substrates

For such as immunohistochemical staining or immunoblotting, substrates arising precipitate products with peroxidase.

AEC (= 3-Amino-9-ethylcarbazole)	1g / 5g	[A2167]
4-CN (= 4-Chloro-1-naphthol)	1g / 5g	[C2291]
DAB (= 3,3'-Diaminobenzidine)	1g / 5g	[D3756]
DAB·4HCl (= 3,3'-Diaminobenzidine Tetrahydrochloride Hydrate)	1g / 5g	[D3757]
***o*-Dianisidine** *2	1g / 5g	[D3864]
***o*-Dianisidine Dihydrochloride** *2	1g / 5g	[D3893]
DMPD·2HCl (= *N*,*N*-Dimethyl-1,4-phenylenediamine Dihydrochloride) *3	1g / 5g	[D3931]
1-Naphthol *3	1g / 5g	[N0864]

*2 : By combinating N0864 and D3931 *3 : Used together with C2291

Alkaline Phosphatase Substrates

4-Nitrophenyl Phosphate (Ready-to-use solution) [for ELISA]
(= pNPP (Ready-to-use solution)) 100mL [N1109]

Application

1. Add 100µL of pNPP solution [N1109] to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. To terminate the reaction, add 100 µL of 1N NaOH solution [S0542] to each well.
4. Within 1 hour from start the reaction, measure the absorbance of each well at 405 nm.

Figure. An example of use by the above method

NBT / X-Phosphate *p*-Toluidine Salt Solution (50X) [for Western blotting] 5mL [N1113]

Application

1. Incubate a blotting membrane with an ALP-conjugated antibody and then wash the membrane.
2. Dilute the solution [N1113] to 1X before use.
3. Incubate the washed membrane with 1X NBT / X-Phosphate *p*-Toluidine Salt solution until color development.
4. Add deionized water to stop color development.

Soluble Substrates

4-Nitrophenyl Phosphate Disodium Salt Hexahydrate	1g / 5g [D4005]
4-Nitrophenyl Phosphate Di(tris) Salt Hydrate	5g / 25g [N0422]
1-Naphthylphosphoric Acid Monosodium Salt Monohydrate	1g / 5g / 25g [N0452]
1-Naphthylphosphoric Acid Disodium Salt Hydrate	1g / 5g [P0263]

Precipitate Substrates

For such as immunohistochemical staining or immunoblotting, substrates arising precipitate dyes with alkaline phosphatase.

Fast Blue RR Salt	5g / 25g [B0785]
X-Phosphate *p*-Toluidine Salt	100mg / 1g [B1239]
Blue Tetrazolium	1g / 5g [B3581]
Nitro Blue Tetrazolium (= NBT)	100mg / 1g [D0844]
Iodonitrotetrazolium Chloride (= INT)	100mg / 1g [I0781]
Tetranitro Blue Tetrazolium (= TNBT)	100mg / 1g [T0250]

Chemiluminescent Reagent for Western Blotting

Chemiluminescence HRP Substrate Solution Kit [for Western Blotting] 1kit [C4087]

C4087 is a chemiluminescent detection reagent for horseradish peroxidase (HRP)-labeled probes bound to proteins on membranes. The two-component type is used by mixing 1:1 immediately before use. Areas in the picogram range can be detected.

Example for use

1. Allow C4087 to reach room temperature.
2. Add HRP-conjugated antibody to the blotted membrane and wash.
3. Mix equal volumes of Solution A and Solution B.
   * Approximately 0.1 mL of mixture per 1 cm^2 of membrane.
4. Blot off excess wash buffer from the membrane.
5. Place the membrane on plastic wrap or a clear polyethylene sheet.
6. Pour the mixture over the membrane.
7. Allow to react for 60 seconds at room temperature.
8. Remove excess mixture.
9. Detect chemiluminescence.
   * Adjust exposure time according to signal intensity.

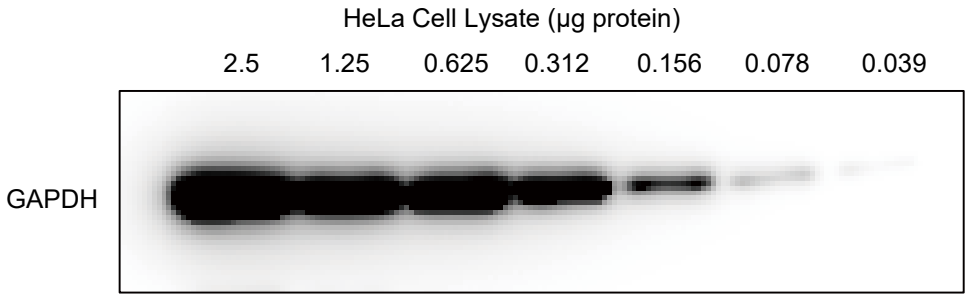


Figure. Membrane luminesced with C4087

HeLa Cell Lysate: 2.5 - 0.039 µg/lane (2x step dilution)
Membrane: PVDF membrane
Blocking Buffer: 1% BSA/TBS-T
Primary Antibody: Anti-GAPDH (Mouse IgG)
Secondary Antibody: Goat anti-Mouse IgG HRP
Detection: CCD Imager
Exposure Time: 120 seconds

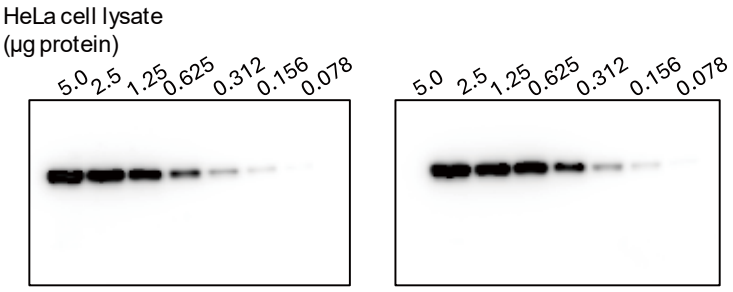
Antibody Stripping Solution for Western Blotting

Western Blot Stripping Buffer [for Biochemical Research] 250mL [W0024]

W0024 is used to strip antibodies from membranes that have undergone chemiluminescence detection. The antigen is retained on the membrane because the procedure is performed under mild conditions. This allows chemiluminescence detection to be repeated with a different antibody.

Example for use

1. Separate 2-fold step dilutions of Hela cell lysate (5.0 - 0.078 µg/lane) by SDS-PAGE.
2. After Western blotting, detect antibodies using chemiluminescence reagents (Figure A).
3. Immerse the membrane in TBS-T and shake for 10 minutes. Repeat this procedure twice.
4. Immerse the membrane in **W0024** and shake for 30 minutes at room temperature (Figure B).
5. Immerse the membrane in TBS-T and shake for 10 minutes. Repeat three times.
6. Start the blocking procedure again and detect the new antibody by chemiluminescence (Figure C).



Membrane: PVDF membrane
Blocking Buffer: 1% BSA/TBS-T
Wash Buffer: TBS-T
Primary Antibody: Anti-αTubulin (Rabbit IgG)
Secondary Antibody: Goat anti-Rabbit IgG HRP
Detection: CCD Imager
Exposure time: 60 seconds

Figure A. First detection. Detection by binding of Anti-αTubulin Antibody (Rabbit IgG).

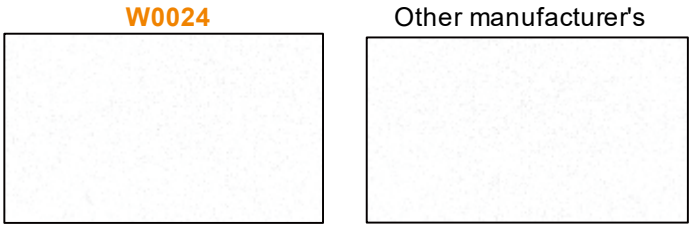
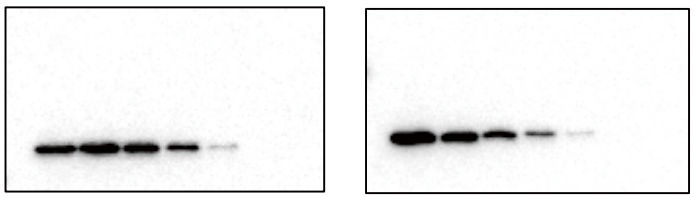


Figure B. Antibody removal with **W0024**



Blocking Buffer: 1% BSA/TBS-T
Wash Buffer: TBS-T
Primary Antibody: Anti-GAPDH (Mouse IgG)
Secondary Antibody: Goat Anti-Mouse IgG HRP
Detection: CCD Imager
Exposure time: 60 seconds

Figure C. Second detection. Detection by re-blocking the stripped membrane and binding Anti-GAPDH Antibody (Mouse IgG)

Protein Staining Reagent

Silver Stain Kit [for Electrophoresis] 1kit [I1309]

Silver staining is a commonly-used method for the detection of proteins and DNA in polyacrylamide gels after electrophoresis. In this method, silver ions are bound to proteins and DNA present in the gel and reduced, resulting in stained bands. Silver staining is more sensitive than Coomassie Brilliant Blue (CBB) staining; it can detect down to nanogram amounts of protein.

Usage Example

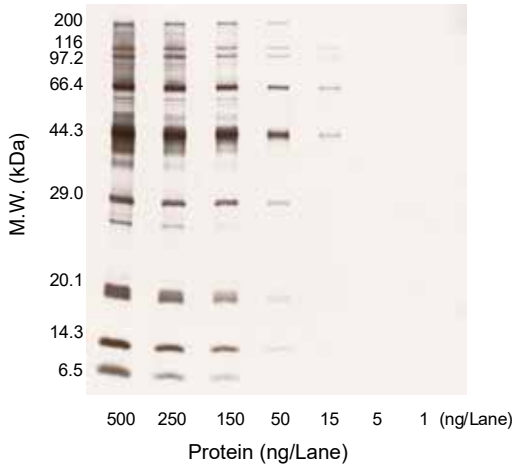


Figure. Protein molecular weight markers were diluted, run on an acrylamide gel, and stained

1. Prepare Fixing Solution, Staining Solution, Developer Solution, and Stop Solution by diluting the supplied solutions 100-fold.
2. In a clean tray, submerge the gel in Fixing Solution, and allow to incubate with shaking for 10 minutes.
3. Remove Fixing Solution, and wash gel in deionized water with shaking for 10 minutes. (Repeat a total of three times)
4. Remove deionized water and replace with Staining Solution. Incubate with shaking for 5 minutes.
5. Remove Staining Solution and replace with deionized water. Incubate with shaking for 30 seconds.
6. Remove deionized water and replace with Developer Solution. Incubate with shaking for 30 seconds.
7. Replace old Developer Solution with fresh solution. Incubate with shaking until developed bands appear.
8. Remove Developer Solution and replace with Stop Solution. Incubate with shaking for 10 minutes.
9. Remove Stop Solution, and wash gel a total of three times with deionized water, incubating with shaking for 5 minutes each wash.

Related Products

2X SDS-PAGE Sample Buffer (2-Mercaptoethanol free) [for Electrophoresis]	25mL	[B5834]
4X SDS-PAGE Sample Buffer (2-Mercaptoethanol free) [for Electrophoresis]	20mL	[B6104]
6X Sample Buffer (2-Mercaptoethanol free) [for Electrophoresis]	10mL	[B6105]
Pyrogallol Red [for Protein Research]	1g	[P1976]
Streptomycin Sulfate [for Protein Research]	5g / 25g	[S0834]
Acrylamide Monomer [for Electrophoresis]	25g / 500g	[A1132]
30% Acrylamide / Bis-acrylamide (29:1) [for Electrophoresis]	250mL	[A3217]
30% Acrylamide / Bis-acrylamide (37.5:1) [for Electrophoresis]	250mL	[A3218]
Acid Black 1 [for Electrophoresis]	5g	[A2097]
Ammonium Peroxodisulfate [for Protein Research]	5g / 25g	[A2098]
Coomassie Brilliant Blue G-250 [for Electrophoresis]	5g	[B3193]
Coomassie Brilliant Blue R-250 [for Electrophoresis]	5g	[B3194]
Bromophenol Blue Sodium Salt [for Electrophoresis]	1g	[B3195]
Sodium Deoxycholate [for Electrophoresis]	25g	[D1820]
DL-Dithiothreitol [for Electrophoresis]	1g / 5g	[D3647]
Glycerol [for Electrophoresis]	1g	[G0316]
Glycine [for Electrophoresis]	25g / 500g	[G0317]
Gel Negative Stain kit [for Electrophoresis]	1kit	[G0615]
***N,N'*-Methylenebisacrylamide** [for Electrophoresis]	25g / 100g	[M0506]
2-Mercaptoethanol [for Electrophoresis]	5g / 25g	[M1948]
Sodium Dodecyl Sulfate (=SDS) [for Electrophoresis]	25g / 500g	[S0588]
***N,N,N',N'*-Tetramethylethylenediamine (=TEMED)** [for Electrophoresis]	5g / 25g	[T2515]
Tris(hydroxymethyl)aminomethane (=Tris-Base) [for Electrophoresis]	25g / 500g	[T2516]

Protein Determination Reagents

Pyrogallol Red (Ready-to-use solution) [for Protein determination]

100mL [P2575]

Application

1. Prepare standard protein solutions with a series of dilutions.
2. Mix **P2575** with unknown protein samples, standard protein solutions and distilled water according to Table 1.
3. Incubate for 30 minutes at room temperature.
4. Measure absorbance at 600 nm.
5. Prepare a standard curve by plotting the absorbance data measured in step 4 after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

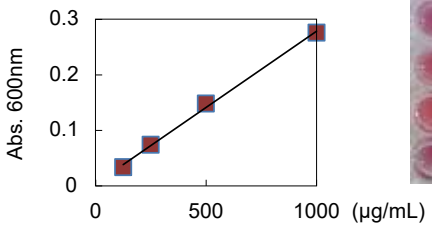
Table 1 : Volume for test tube or micro plate assay

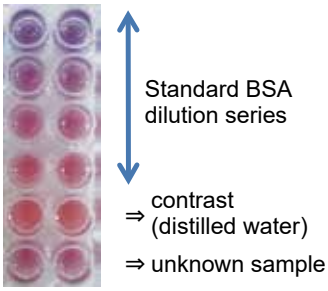
Assay	test tube	micro plate
Measurement range	0.1 -1.0 mg/mL	0.1 -1.0 mg/mL
Sample solution or protein standard*	50 µL	10 µL
P2575	1 mL	200 µL

*P2575 requires the standard protein solution (such as BSA).

Example for use: in a microplate

1. Prepare four dilution series of standard protein solutions from the concentration at 1000 µg/mL by doubling dilution.
2. Mix 200 µL of **P2575** with 10 µL each of a protein sample at an unknown concentration, the standard protein solution and distilled water in a 96 microplate.
3. Incubate for 30 minutes at room temperature, measure absorbance at 600 nm, and prepare a standard curve.





Bradford Assay Solution (Ready-to-use) [for Protein determination]

500mL [B5702]

Application

1. Prepare standard protein solutions with a series of dilutions.
2. Mix **B5702** with unknown protein samples, standard protein solutions and distilled water according to Table 2.
3. Incubate for 5 minutes at room temperature.
4. Measure absorbance at 600 nm.
5. Prepare a standard curve by plotting the absorbance data measured in step 4 after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

Table 2 : Volume for test tube or micro plate assay

Assay	test tube	micro plate	micro assay
Measurement range	0.1 -1.0 mg/mL	0.1 -1.0 mg/mL	0.1 -25 µg/mL
Sample solution or Protein standard*	50 µL	10 µL	500 µL
B5702	1 mL	200 µL	500 µL

*B5702 requires the standard protein solution (such as BSA).

Ordering and Customer Service

TCI AMERICA
Tel : 800-423-8616 / 503-283-1681
Fax : 888-520-1075 / 503-283-1987
E-mail : Sales-US@TCIchemicals.com

TCI EUROPE N.V.
Tel : +32 (0)3 735 07 00
Fax : +32 (0)3 735 07 01
E-mail : Sales-EU@TCIchemicals.com

TCI Deutschland GmbH
Tel : +49 (0)6196 64053-00
Fax : +49 (0)6196 64053-01
E-mail : Sales-DE@TCIchemicals.com

Tokyo Chemical Industry UK Ltd.
Tel : +44 (0)1865 78 45 60
E-mail : Sales-UK@TCIchemicals.com

梯希爱(上海)化成工业发展有限公司
Tel : 800-988-0390 / 021-67121386
Fax : 021-6712-1385
E-mail : Sales-CN@TCIchemicals.com

Tokyo Chemical Industry (India) Pvt. Ltd.
Tel : 1800 425 7889 / 044-2262 0909
E-mail : Sales-IN@TCIchemicals.com

TOKYO CHEMICAL INDUSTRY CO., LTD.
Tel : +81 (0)3-5640-8878
E-mail : globalbusiness@TCIchemicals.com