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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

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Isotype</th>
<th>Size</th>
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<tbody>
<tr>
<td>Anti-GM₁ Monoclonal Antibody</td>
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<tr>
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<tr>
<td>Anti-GM₃ Monoclonal Antibody</td>
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<td>Anti-GD₁a Monoclonal Antibody</td>
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<tr>
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### Anti-Sulfated Glycan Antibodies

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<th>Isotype</th>
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<tr>
<td>Anti-6-sulfo LacNAc Monoclonal Antibody (AG105)</td>
<td>Mouse IgM</td>
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<tr>
<td>Anti-6,6’-disulfo LacNAc Monoclonal Antibody (L4L4-8)</td>
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<td>Anti-Sialyl 6-sulfo Lewis X Monoclonal Antibody (G152)</td>
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Antibodies and Related Reagents

### Anti-Glycosaminoglycan Antibodies

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<td>Anti-Perlecan Monoclonal Antibody (HK-102)</td>
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### Anti-Blood Group Antigen Antibodies

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<td>Mouse IgG3</td>
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<td>Anti-Sialyl Lewis A Monoclonal Antibody (2D3)</td>
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<td>Mouse IgM</td>
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### 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.

<table>
<thead>
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<td>HSA-GM1 Pentasaccharide</td>
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<td>HSA-Globo-H</td>
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<td>HSA-L1-L1</td>
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<td>[H1782]</td>
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</tbody>
</table>

### Synthetic Carbohydrate Chains

- **SSEA-1-PrNH$_2$** [S0946]
- **Sialyl Lewis X–Lactose** [S0849]

Feel free to contact us. Using advanced proprietary technologies, we synthesize a wide range of sugar chains for daily research.
**Anti-NeuGc Polyclonal Antibodies**

*N*-Acetylneuraminic Acid (NeuAc) and *N*-Glycolyneuraminic 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**

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 NeuGca(2-3)Gal and NeuGca(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.

- Neu5Aca(2-3)Galβ MP Glycoside [N0791]
- Neu5Aca(2-6)Galβ MP Glycoside [N0792]
- Neu5Gca(2-3)Galβ MP Glycoside [N0793]
- Neu5Gca(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 min) with isotype control (black line) or anti-NeuGc polyclonal antibody R-PE conjugate [A3360] (red line) adjusted to 0.1 µg/mL. Afterward, it was measured using a flow cytometer.
**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 antibody shows the same high specificity compared with an anti-αGal monoclonal antibody**

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 min) with isotype control (black line) or anti-αGal polyclonal antibody R-PE conjugate [A3354] (red line) adjusted to 0.1 μg/mL. Afterward, it was measured using a flow cytometer.
**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.

**Protein A Products**

**Protein A Recombinant, expressed in *Escherichia coli*** 5mg/vial [P2366]

**Protein A Biotin Conjugate** 1mg/vial [P2407]

**Protein A HRP Conjugate** 0.2mg/vial [P2467]

**Protein A Agarose** 2mL/vial [P2461]

Protein A is a type I membrane protein produced by several strains of *Staphylococcus aureus*. It has high-affinity binding sites for IgGs obtained from various species such as humans, rabbit, mouse, and bovine. Protein A supported by agarose resin is prepared using a covalent coupling method and can be applied to the purification of IgGs. By using [P2461], human IgGs can be eluted under milder conditions (such as at pH 4.0) compared to using other resins with conventional eluting protocols.
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]

**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]

---

**Anti-Cell Marker Antibodies**

**Mouse Anti-NeuN Monoclonal Antibody**

0.1mg/vial [M3586]

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, 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.
Antibodies and Related Reagents

**Anti-Endo-M Antibodies**

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

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

**Enzymes which Transfers the Intact Oligosaccharides**

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

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

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

**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]
## 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, 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]

### Anti-Human IgG
- **Anti-Human IgG Fc C-terminus Monoclonal Antibody**: 0.1mg/vial [A3277]
- **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-Eu³⁺ Conjugate**: 0.1mg/vial [S0993]
- **Streptavidin R-PE Conjugate**: 0.1mg/vial [T3885]
- **Streptavidin Maleimide Conjugate**: 0.5mg/vial [T3531]
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 IgG2ax 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.

<table>
<thead>
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<th>Fluorescence</th>
<th>Quantity</th>
<th>Catalog Number</th>
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<td>Red Fluorescence</td>
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<td>G0506</td>
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<tr>
<td>Streptavidin DTBTA-Eu³⁺ Conjugate</td>
<td></td>
<td>0.1mg/vial</td>
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<tr>
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<td>Blue Fluorescence</td>
<td>5mg</td>
<td>A2412</td>
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<tr>
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<td>Blue Fluorescence</td>
<td>0.2mL x 5vial</td>
<td>D5888</td>
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<tr>
<td>Bisbenzimide H 33258 Hydrate</td>
<td>Blue Fluorescence</td>
<td>25mg</td>
<td>H1343</td>
</tr>
<tr>
<td>Bisbenzimide H 33258 (1mg/mL in Water)</td>
<td>Blue Fluorescence</td>
<td>0.2mL x 5vial</td>
<td>B6236</td>
</tr>
</tbody>
</table>

*Some products are unavailable in the Americas and China.

*The high-sensitivity detection of DTBTA-Eu³⁺ labeled probes requires time-resolved fluorometry.
Antibodies and Related Reagents

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

Highly-sensitive Detection Probes for Time-resolved Fluorometry

Goat Anti-Mouse IgG DTBTA-Eu$^{3+}$ Conjugate  
Goat Anti-Rabbit IgG DTBTA-Eu$^{3+}$ Conjugate  
Streptavidin DTBTA-Eu$^{3+}$ Conjugate  

No cross talk of excitation light  
- Excitation wavelength $E_x$ max : 335 nm  
- Emission wavelength $E_m$ 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 ($\tau = 1.02$ 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

<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 $\mu$g/mL. After incubation, measure the fluorescence intensity on a plate reader. DTBTA-Eu$^{3+}$: excitation=340 nm, emission=620 nm. Lag Time : 450 psec

Anti-DTBTA-Eu$^{3+}$ Antibody  
Anti-DTBTA-Eu$^{3+}$ Rabbit Polyclonal Antibody  
DTBTA-Eu$^{3+}$ Labeling Reagent

ATBTA-Eu$^{3+}$
Fluorescent Organosilica Particles

Organosilica FITC (100nm Diam.)
Organosilica Rhodamine B (100nm Diam.)

2mg [O0561]
2mg [O0573]

**Advantages**

- **Wavelength:** $\text{Ex}_{\text{max}}$ 492 nm, $\text{Em}_{\text{max}}$ 523 nm ([O0561])
  $\text{Ex}_{\text{max}}$ 556 nm, $\text{Em}_{\text{max}}$ 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**

- **Organosilica FITC [O0561] spectrum**
  - Excitation
  - Emission
- **Organosilica Rhodamine B [O0573] spectrum**
  - Excitation
  - Emission

**Application**

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 Goat anti Mouse IgG antibody](image)

**Labeling with Protein A**

![Labeling with Protein A](image)

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.

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

13
**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-hydroxysuccinimidy (NHS) moiety and can be used to readily label proteins and peptides that have an amino group (-NH₂).
- **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 ºC.
4) To quench the reaction, add 200 μL of 100mM Tris-HCl buffer pH 7.5.
5) Incubate for 1 hour at 37 ºC.

*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

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 2-Iminothiolane.

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-Acetylthioylglycolate) 1g / 5g [S0431]
- SATP (= N-Succinimidyl 3-(Acetylthio)propionate) 100mg [S0859]
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.

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**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**

![Graph showing the relationship between Anti-6xHis Antibody concentration and Abs. 450nm](image)

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.

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Please inquire for pricing and availability of listed products to our local sales representatives.
**Cell Proliferation Assay Reagents**

**Resazurin** *(Ready-to-use solution) [for Cell proliferation assay]*

**Mechanism**

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.

**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 spectrophotometer 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.

---

**Cell Staining Reagents**

**Methylene Blue Solution** *(Methanol Solution) [for Cell Staining]*

**Application**

1) Culture cells in a 6-well plate.
2) Remove medium from the plate and wash it with PBS(-) twice.
3) Remove PBS(-) from it, add 1 mL of M2392 and stain cells for 15 minutes.
4) Remove M2392 from it and wash it with deionized water twice.

*For measurement of cell proliferation, it is best to add resazurin during the log phase of growth.*

---

**Figure. NIH/3T3 cells stained by the above method**

Please adjust staining time and volume according to cells. Because some cells need to be fixed separately, preliminary tests should be performed.
Antibodies and Related Reagents

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 10⁶ 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.

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.

Please inquire for pricing and availability of listed products to our local sales representatives.
**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](image)

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](image)

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

<table>
<thead>
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<th>kDa</th>
<th>M</th>
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<td>32</td>
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</tbody>
</table>

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]
Antibodies and Related Reagents

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 H₂O₂)

Substrates generating soluble dyes for determining hydrogen peroxidase (H₂O₂) 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 Hydrate** (= Sodium 3-[Ethyl(m-tolyl)amino]-2-hydroxy-1-propanesulfonate Hydrate) *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 / 1g [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 combining N0864 and D3931  *3 : Used together with C2291

Please inquire for pricing and availability of listed products to our local sales representatives.
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]  
**Naphthol AS-TR Phosphate**  
200mg [C2250]  
**Nitro Blue Tetrazolium (= NBT)**  
100mg / 1g [D0844]  
**Iodonitrotetrazolium Chloride (= INT)**  
100mg / 1g [I0781]  
**Tetranitro Blue Tetrazolium (= TNBT)**  
100mg / 1g [T0250]
Protein Staining Reagent

Coomassie Brilliant Blue G-250 (Ready-to-use solution) [for Electrophoresis] 500mL [C3488]

**Application**

1) After electrophoresis, wash the gel with deionized water for 5 minutes three times.
2) Remove the water used for washing, add C3488 till the gel is soaked, and stain the gel for 1 hour while shaking gently at room temperature.
3) Remove the staining solution, destain the gel with deionized water for 1 hour and check it.
4) If the background is high, destain the gel with deionized water overnight at room temperature.

![Figure: Proteins stained by the above method (destained overnight)](image)

**Related Products**

- **2X SDS-PAGE Sample Buffer (2-Mercaptoethanol free)** 25mL [B5834]
- **4X SDS-PAGE Sample Buffer (2-Mercaptoethanol free)** 20mL [B6104]
- **6X Sample Buffer (2-Mercaptoethanol free)** 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]
- **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]