New

LIFE SCIENCE

Xanthine / Hypoxanthine Assay Kit

Xanthine / Hypoxanthine Assay Kit

1kit [X0094]

The two purine bases xanthine and hypoxanthine can be found in most tissues and biological fluids. Xanthine is produced during the digestion of purine to uric acid by the action of xanthine oxidase. This kit measures the xanthine / hypoxanthine concentration of biological samples upon comparison with a xanthine standard solution.

Advantages

- Enables sensitive measurement of Xanthine / Hypoxanthine maximum to 400 µM
- Quick procedure with results within 30 minutes
- Suitable for various biological samples

Kit Components

• 10 x Assay Buffer	1vial
• Advanced Enzyme II Solution	1vial
Advanced Substrate Solution	1vial
• Enzyme Solution	1vial
• 20mM Xanthine Standard Solution	1vial

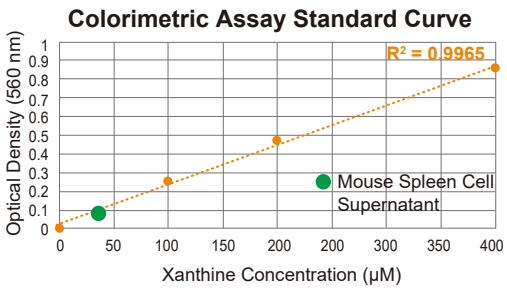
Each vial is sufficient for 96 tests.

Example: Xanthine / Hypoxanthine quantification by colorimetric assay

‡ Xanthine / Hypoxanthine quantification from 20 µM to 400 µM can be tested via the colorimetric assay.

1. **Prepare 1 x Assay Buffer**
   Mix 20 mL of 10 x XO Assay Buffer with 180 mL of distilled water.
2. **Prepare standard curve**
   2.1. Mix 10 µL of Xanthine Standard Solution with 40 µL of 1 x Assay Buffer to make a 4 mM Xanthine.
   2.2. Prepare the xanthine standard solutions shown in the right table.
3. **Prepare standards and samples**
   Aliquot 50 µL of standards and samples to separate wells of a 96 well plate.
4. **Prepare reaction mixture**
   Mix an appropriate amount of each reagent for the number of wells to be assayed. The volumes below represent the amount of each reagent needed per reaction per well.
   - Enzyme Solution : 1 µL
   - Advanced Substrate Solution : 1 µL
   - Advanced Enzyme II Solution : 1 µL
   - 1 x Assay Buffer : 47 µL
5. **Start reaction**
   Add 50 µL of reaction mixture (prepared in step 4) to each well and mix well. Incubate the reaction at room temperature in the dark for 30 minutes.
6. **Measure Optical Density (OD)**
   Measure OD at a wavelength between 550 - 580 nm.
7. **Calculate activity**
   7.1. Plot standards to obtain a standard curve and calculate the slope.
   7.2. Use the slope, along with the OD value of the sample to calculate xanthine / hypoxanthine concentration based on the following formula:

No.	4 mM Xanthine	1 x Assay Buffer	Final Conc. (µM)
1	0 µL	200 µL	0
2	6 µL	194 µL	120
3	12 µL	188 µL	240
4	20 µL	180 µL	400



$$\text{Xanthine / Hypoxanthine Concentration (µM)} = \frac{OD_{Sample} - OD_{Blank}}{\text{Slope}} \times \text{Dilution ratio}$$

[Experimental Data]
Slope = 0.002194, $OD_{\text{Mouse Spleen Cell Supernatant}} - OD_{Blank}$ = 0.0788, Dilution ratio = 1
Based on the above data, the Xanthine / Hypoxanthine contained in Mouse Spleen Cell Supernatant is 35.92 µM.
(Measurement condition : room temperature, pH 7.5)

Example: Xanthine / Hypoxanthine quantification by fluorometric assay

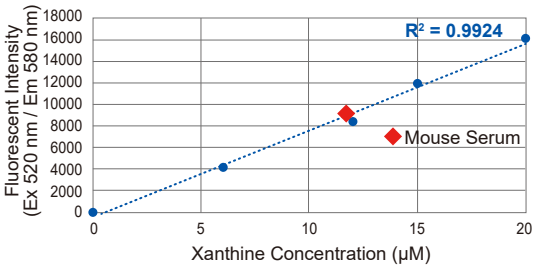
‡ Xanthine / Hypoxanthine quantification up to 20 µM can be tested via the fluorescence assay.

1. **Prepare 1 x Assay Buffer**
   Mix 20 mL of 10 x XO Assay Buffer with 180 mL of distilled water.
2. **Plot standard curve**
   2.1. Mix 2 µL of 20 mM Xanthine Standard Solution with 198 µL of 1 x XO Assay Buffer to make a 200 µM xanthine solution.
   2.2. Prepare the xanthine standard solutions shown in the right table.
3. **Prepare standards and samples**
   Aliquot 50 µL of standards and samples to separate wells of a 96 well plate.
4. **Prepare reaction mixture**
   Mix an appropriate amount of each reagent for the number of wells to be assayed. The volumes below represent the amount of each reagent needed per reaction per well.
   - Enzyme Solution : 1 µL
   - Advanced Substrate Solution : 1 µL
   - Advanced Enzyme II Solution : 1 µL
   - 1 x Assay Buffer : 47 µL
5. **Start reaction**
   Add 50 µL of reaction mixture (prepared in step 4) to each well and mix well. Incubate the reaction at room temperature in the dark for 30 minutes.
6. **Measure Fluorescent intensity (F)**
   Measure F under the condition of λ_{ex} = 520 - 550 nm and λ_{em} = 585 - 595 nm.
7. **Calculate activity**
   7.1. Plot standards to obtain a standard curve and calculate the slope.
   7.2. Use the slope, along with the fluorescence intensity value of the sample to calculate xanthine / hypoxanthine concentration based on the following formula:

$$\text{Xanthine / Hypoxanthine Concentration (µM)} = \frac{F_{Sample} - F_{Blank}}{\text{Slope}} \times \text{Dilution ratio}$$

No.	200 µM Xanthine	1 x Assay Buffer	Final Conc. (µM)
1	0 µL	200 µL	0
2	6 µL	194 µL	6
3	12 µL	188 µL	12
4	20 µL	180 µL	20

Fluorometric Assay Standard Curve



[Experimental Data]
Slope = 773.7638, $F_{Mouse\ Serum} - F_{Blank}$ = 9091, Dilution ratio = 1
Based on the above data, the Xanthine / Hypoxanthine contained in mouse serum is 11.75 µM.
(Measurement condition : room temperature, pH 7.5)

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