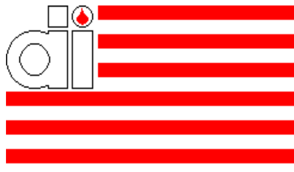

fluorescein conjugated high molecular weight human urokinase

Product No. 124FITC Lot No. 010622

Description

High molecular weight (two chain) urokinase (M_r 54,000) from human urine conjugated with fluoroscein isothiocyanate for use in flow cytometric applications and immuinoflourescent cell and tissue staining.

Presentation

Plastic cyro-tubes each containing 25 µg of FITC conjugated urokinase, frozen solution, 50 µL in PBS, 1% BSA. No preservatives are added to this preparation.

Properties

Prior to conjugation urokinase of specific activity approximately 80,000 IU/mg. High molecular weight form at about 95% low molecular weight form at about 5%. Following carefully FITC conjugation, the functional properties appear to be fully preserved.

Protocol

See reverse side of this data sheet.

Fluorescein/Protein Molar Ratio: 5.6

Storage

Store in the dark at -20°C or colder. This product must always be stored at -20°C as it readily degrades to the low molecular weight form. Avoid freeze-thaw cycles.

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500 West Avenue, P.O. Box 110215, Stamford, CT. 06911-0215 Tel. (203) 602-7777 • Fax. (203) 602-2221

Macrophage Staining for Flow Cytometry

The following protocol stains macrophages for human urokinase receptor using Product No. 124FITC. All solutions (PBS, staining buffer, etc.) plus a rack with microcentrifuge tubes should be kept on ice at all times.

Remove culturing medium from monolayer and place cell culture plates on ice. Wash the monolayer with PBS and add 1 mL of PBS to keep the cells moist. Gently remove the cells using a rubber policeman and rinse the policeman with PBS in the petri dish. With a pipette, place the entire cell suspension in a chilled centrifuge tube (approximately 5 mL).

1. Centrifuge at 1000 - 3000 rpm to pellet cells and wash with staining buffer.
2. Resuspend cells in 0.05M glycine-HCl, 0.1M NaCl, pH 3.0 to a concentration of 1×10^6 cells in 0.4 mL solution.
3. Gently vortex cells for 3 minutes.
4. Quench with 0.5M HEPES, 0.1M NaCl, pH 7.5.
5. Centrifuge to pellet cells, wash 2 times with staining buffer and resuspend in 0.3 mL of cold staining buffer. The ideal concentration is 1 x 106 cells in 0.1 mL buffer.
6. Divide the sample among 3 microcentrifuge tubes, 0.1 mL per tube. These 3 tubes serve as follows:

   Tube A: Negative Control
   Tube B: Non-specific Binding Determination - Addition of FITC-HMW uPA and excess uPA
   Tube C: Test Sample - Addition of FITC-HMW uPA only.
7. Add 0.54 µg of uPA to tube B and incubate on ice for 30 minutes. This is equivalent to a 0.1 µM uPA concentration.
8. Add 8.1 ng of FITC-HMW uPA, No. 124FITC (3.25 µL of 1/100 dilution) to tubes B and C and incubate on ice for 30 minutes. This is equivalent to a 1.5 nM FITC-HMW uPA concentration.
9. Fill the tube with staining buffer, centrifuge to pellet, decant supernatant, vortex pellet and wash again with buffer.
10. Wash the tube and vortex the pellet a final time and resuspend the cells in 2% formaldehyde in PBS (fixative).

Staining Buffer: 0.15M PBS, 1% BSA or FCS

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