Chondrex, Inc.

Tetramethylrhodamine isothiocyanate -Dextran, 70 kDa

Catalog # 4014

For Research Use Only - Not Human or Therapeutic Use

DESCRIPTION:	Tetramethylrhodamine isothiocyanate (TRITC) labeled dextran
APPLICATION:	Use to assess the permeability of semi-permeable membranes either *in vivo* or *in vitro* (1-4). Refer to the protocols below. Note: TRITC-dextran can be used simultaneously with FITC-dextran (Catalog # 4009 or 4013) as fluorescence occurs at different wavelengths.
QUANTITY:	5 ml
FORM:	25 mg/ml solution in PBS
MOLECULAR WEIGHT:	70 kDa
FLUORESCENT PROPERTIES:	Excitation: 550 nm, Emission: 572 nm
STORAGE TEMPERATURE:	4ºC in the dark
STABILITY:	1 year

IN VIVO PROTOCOL:

1. Fast mice 4 hours before oral feeding and for the duration of the experiment.
2. Feed 20 ml/kg by oral gavage.
3. Maintain fasting conditions and wait 3 hours (may vary depending on individual animals).
4. Collect blood by retro-orbital bleeding, then spin and collect plasma. Dilute plasma 1:2 (or more) with PBS.

   Note 1: Protein in the samples may interfere with and reduce the fluorescence intensity (FI). Therefore, in order to accurately determine TRITC-dextran permeability, the plasma to PBS ratio must be consistent throughout all the samples.

   Note 2: A standard curve made from serial dilutions of the stock TRITC-dextran can be used for qualitative studies. See * Preparing Standards on the next page.

6. Transfer 50 or 100 µl of diluted standards and samples to a black 96-well plate and read in a fluorescence reader.

   Settings for Reading: Excitation: 550 nm/Emission: 572 nm
   Wavelength Bandwidth (Excitation and Emission): 9 nm
   Gain: Auto or set 80,000 equivalent to 12.5 µg/ml

IN VITRO PROTOCOL:

A protocol for *in vitro* studies will vary due to the types of cultured cells, culture systems, purpose of study, etc. Please use your own optimized protocol or contact support@chondrex.com for more information.

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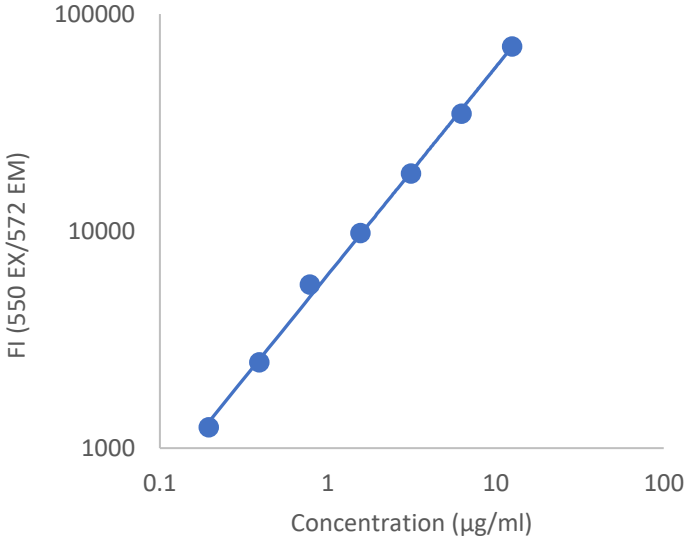
***PREPARING STANDARDS**

A standard range of 12.5 to 0.2 µg/ml is recommended. To prepare standard dilutions, use a diluent with the same ratio of normal mouse plasma to PBS as the samples. For example, if using a 1:2 dilution for sample dilution (*in vivo* protocol, Step 4), prepare 33% normal mouse plasma in PBS (300 µl plasma with 600 µl PBS) as a diluent.

1. 10 µl of 25 mg/ml TRITC-Dextran with 990 µl of PBS (250 µg/ml).
2. 12.5 µl of the diluted TRITC-Dextran with 237.5 µl of 33% normal mouse plasma in PBS (12.5 µg/ml).
3. Mix 125 µl of the 12.5 µg/ml solution with an equal volume of 33% mouse normal plasma (6.3 µg/ml).
4. Repeat 5 times for the 3.1, 1.6, 0.8, 0.4, and 0.2 µg/ml standard solutions.
5. Transfer 50 or 100 µl of diluted standards and samples to a black 96-well plate and read on a fluorescence reader.
6. Subtract the FI blank values (33% normal mouse plasma in PBS) from the FI values of the standards and samples.
7. Plot the FI values of the standards against the µg/ml of the TRITC-Dextran standards. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve.

Figure 1. A Typical Standard Curve



(µg/ml)	FI Values	Corrected
12.5	77804	70760
6.25	41765	34721
3.13	25476	18432
1.56	16829	9785
0.78	12706	5662
0.39	9525	2481
0.20	8290	1246
33% Serum/PBS	7044	0

REFERENCES:

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2. J. W. Kim, J. D. Lindsey, N. Wang, R. N. Weinreb, Increased human scleral permeability with prostaglandin exposure. *Invest Ophthalmol Vis Sci* **42**, 1514-1521 (2001).
3. D. B. Pink, W. Schulte, M. H. Parseghian, A. Zijlstra, J. D. Lewis, Real-time visualization and quantitation of vascular permeability *in vivo*: implications for drug delivery. *PLoS One* **7**, e33760 (2012).
4. R. K. Sajja, S. Prasad, L. Cucullo, Impact of altered glycaemia on blood-brain barrier endothelium: an in vitro study using the hCMEC/D3 cell line. *Fluids Barriers CNS* **11**, 8 (2014).

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