Fluorescein Isothiocyanate-Dextran, 4 kDa

Catalog # 4013

For Research Use Only - Not Human or Therapeutic Use

DESCRIPTION:	Fluorescein isothiocyanate (FITC) labeled dextran
APPLICATION:	Use to assess the permeability of semi-permeable membranes either *in vivo* or *in vitro* (1-4). NOTE: FITC-dextran can be used simultaneously with TRITC-dextran (Cat # 4014) as fluorescence occurs at different wavelengths.
QUANTITY:	5 ml
FORM:	25 mg/ml solution in 0.05M phosphate buffered saline
MOLECULAR WEIGHT:	4 kDa
FLUORESCENCE:	Excitation: 490 nm, Emission: 520 nm
STORAGE:	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 FITC-dextran permeability, the plasma to PBS ratio must be consistent throughout all the samples.

   NOTE 2: Chondrex, Inc. recommends making a standard curve from serial dilutions of the stock FITC-dextran for qualitative studies.
5. 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: 490 nm/Emission: 520 nm

   Wavelength Bandwidth (Excitation and Emission): 9 nm

   Gain: Auto or set 80,000 equivalent to 12.5 µg/ml

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

IN VITRO PROTOCOL:

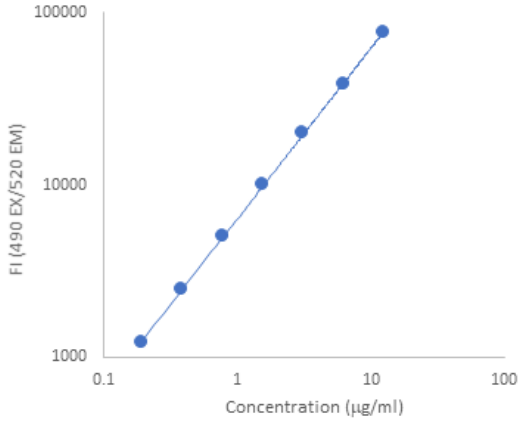
A protocol for *in vitro* studies will vary due to the types of cultured cells, culture systems, purpose of study, etc. Please contact support@chondrex.com for more information about optimization.

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 0.05M phosphate buffered saline (300 µl plasma with 600 µl PBS) as a diluent.

1. 10 µl of 25 mg/ml FITC-Dextran with 990 µl of PBS (250 µg/ml).
2. 12.5 µl of the diluted FITC-Dextran with 237.5 µl of 33% normal mouse plasma in 0.05M phosphate buffered saline (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 0.05M phosphate buffered saline) from the FI values of the standards and samples.
7. Plot the FI values of the standards against the µg/ml of the FITC-Dextran standards. Using a log/log plot will linearize the data (Figure 1).

Figure 1. A Typical Standard Curve for FITC-Dextran



(µg/ml)	FI Values	Corrected
12.5	76281	75850
6.25	39052	38621
3.13	20389	19958
1.56	10414	9983
0.78	5471	5040
0.39	2903	2472
0.20	1632	1201
33% Serum/PBS	431	0

NOTES:

N/A

REFERENCES:

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2. Q. Wang, C. Fang, P. Hasselgren, Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice. *Am J Physiol Regul Integr Comp Physiol* **281**, R1013-23 (2001).
3. 3. W. Huang *et al*., HMGB1 increases permeability of the endothelial cell monolayer via RAGE and Src family tyrosine kinase pathways. *Inflammation* **35**, 350-362 (2012).