D-Xylose Assay Kit

Catalog # 6601

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to evaluate cellular permeability
FORMAT:	96-well ELISA Plate with non-removeable strips
ASSAY TYPE:	Colorimetric Assay
ASSAY TIME:	1 hour
STANDARD RANGE:	20 mM to 0.3 mM
NUMBER OF SAMPLES:	Up to 39 (duplicate) samples/plate
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	Undiluted
CHROMOGEN:	N/A (read at 554 nm)
STORAGE:	Room Temperature
VALIDATION DATA:	Intra-Assay (5.6-8.0%)/Inter-Assay (4.6-6.4%)/Spiking Test (95-104%)
NOTES:	

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INTRODUCTION

The transport of molecules from the gut into the body occurs either in between the cells or through the cells, paracellularly or transcellularly, respectively. Fluorescent-labeled Dextrans can be used to evaluate paracellular permeability, while D-Xylose, a simple monosaccharide, can be used to evaluate transcellular uptake.

In fact, it may be necessary to use two different markers to evaluate permeability. For example, two groups of rats fed total parenteral nutrition supplemented with two different amino acid cocktails showed an increase in fluorescein isothiocyanate (FITC)-labeled dextran permeability in one group over the other, but no discernible difference in D-Xylose uptake between the two groups (1). This data shows that measuring only one mode of transport may result in incorrect conclusions about permeability.

Chondrex, Inc. provides a D-Xylose Assay Kit (Cat # 6601) in a convenient 96-well plate format, as well as FITC-labeled Dextrans (Cat # 4009:4 kDa and Cat # 4013:40 kDa) to evaluate cellular permeability *in-vitro* or *in-vivo*. Rhodamine-labeled Dextran (Cat # 4014: 70 kDa), which fluoresces at a different wavelength than FITC, is also available, permitting the simultaneous use of two different sized Dextrans which can be individually measured. Moreover, Chondrex, Inc. offers an array of mouse and human anti-bacteria and anti-lipopolysaccharides (LPS) antibody ELISA kits as an alternative method for evaluating gut barrier integrity and proper immune function against these environmental factors. Please visit contact support@chondrex.com or www.chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
D-Xylose Standard Solution (66011)	1 vial	40 mM, 100 µl	Room Temperature
D-Xylose (66012)	1 vial	500 mg	Room Temperature
Reagent 1 (66013)	1 vial	200 mg	Room Temperature
Solution A (66014)	1 vial	1.5 ml	Room Temperature
96-Well Plate	1 each	8-well strips x 12	Room Temperature

REAGENTS NOT PROVIDED

1. Glacial Acetic Acid*
2. Concentrated Hydrochloric Acid*

*Please use caution and wear the appropriate protective gear when working with strong acids

IN-VITRO PROTOCOL

Please refer to references 2-5 to establish a working protocol

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IN-VIVO PROTOCOL

1. Fast mice 4 hours before oral feeding and during the experiment.

2. Feed each mouse 200 µl of the D-Xylose solution by oral gavage. The concentration of D-Xylose must be optimized for individual experimental purposes. A 100 mg/ml D-Xylose solution (500 mg D-Xylose in 5 ml PBS) works for most purposes. For rats, feeding 2 ml of a 50 mg/ml solution works for most purposes (1).

3. Maintain fasting conditions and wait 1 hour (time may vary depending on individual animals).

4. Collect blood by retro-orbital bleeding, then spin, and collect serum (plasma). Proceed with D-Xylose Assay or store at -20°C until use.

   NOTE: It is possible to simultaneously evaluate transcellular permeability with D-Xylose and paracellular permeability with fluorescent-labeled Dextrans (Catalog # 4009, 4013, and 4014). However, follow the appropriate protocols, as incubation times for paracellular permeability and transcellular permeability differ. Also, consult the appropriate animal committee for multiple bleedings on the same day.

ASSAY OUTLINE

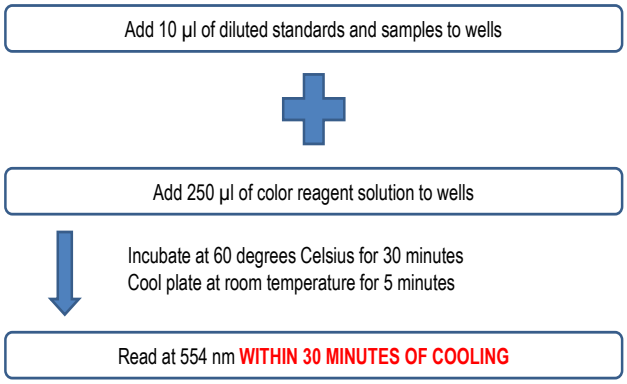
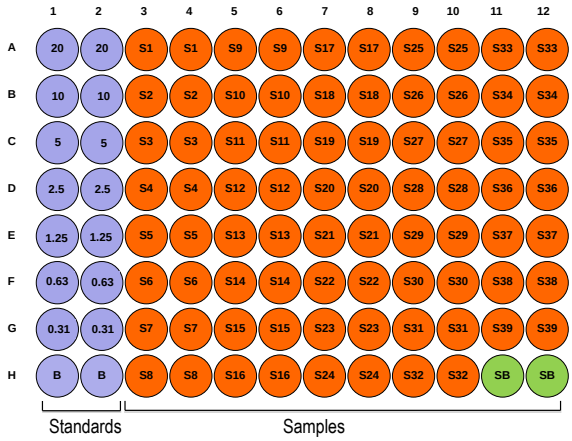


PLATE LAYOUT



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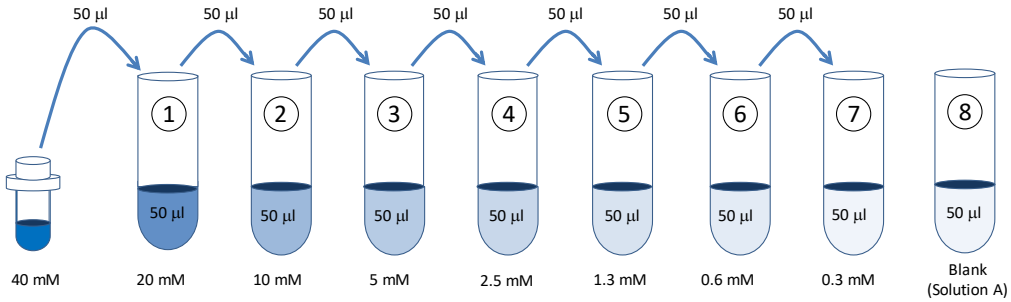
NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

ASSAY PROCEDURE

1. **Prepare D-Xylose Standard:** The recommended standard range is 0.3-20 mM. To dilute the standard, take 50 µl of the D-Xylose Standard (40 mM) and add to 50 µl of Solution A to make a 20 mM D-Xylose standard solution; then serially dilute it with Solution A. For example, mix 50 µl of the standard (20 mM) with an equal volume of Solution A to make a 10 mM solution, and then repeat it five more times to make 5, 2.5, 1.25, 0.63, and 0.31 mM standard solutions (Solution A will be the Blank). The remaining 40 mM Standard Stock solution can be stored at room temperature for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



2. **Prepare Samples:** Serum samples may be used undiluted. If D-Xylose levels in a sample are above 20 mM, then dilute the sample 2-3 times and re-assay.

   NOTE**:** Chondrex, Inc. recommends preparing sample blanks using D-Xylose free serum (plasma) or D-Xylose free culture media because serum or culture media components, such as glucose, may interfere with the assay. Refer to Plate Mapping.

3. **Prepare Color Reagent:** 250 µl of color reagent is required for each well. For example, a 7-point standard, blank, sample blank, and 15 samples, all in duplicate will be 48 wells. Therefore, 12 ml of color reagent is needed. Dissolve 60 mg of Reagent 1 in 12 ml of concentrated acetic acid (AcOH) and 1.2 ml of concentrated hydrochloric acid (HCl). Use caution when working with strong acids.

   NOTE: Prepare the Color Reagent just before use. Do not store the prepared solution for reuse in a future assay.

4. **Add Standards, Samples and Sample Blank:** Add 10 µl of solution A (blank), standards, sample blank, and samples into designated wells.

5. **Add Color Reagent:** Carefully add 250 µl of freshly prepared Color Reagent into each well. Seal the wells with a plate sealer to prevent evaporation during incubation. If only using half the plate, cut the plate sealer in half and save for a future assay. Incubate at 60°C for 30 minutes.

6. **Read Plate:** Remove the plate from the incubator and carefully remove the plate sealer. Allow the plate to cool at room temperature for 5 minutes. Next, read the OD values at 554 nm. Read the plate within 30 minutes of cooling. If the OD values of the samples are greater than the OD value of the highest standard point, dilute the samples 2-3 times and re-assay.

CALCULATING RESULTS

1. Average the duplicate OD values for the blank (and sample blank), standards, and test samples.

2. Subtract the averaged blank OD value from the individual averaged standard, sample, and sample blank OD values. Subtract the averaged sample blank OD value from the averaged sample OD values.

3. Plot the subtracted OD values of the standards against the concentration (mM) of D-Xylose. Using a log/log plot will linearize the data. Figure 1 shows representative OD values of the standard range from 0.3 - 20 mM.

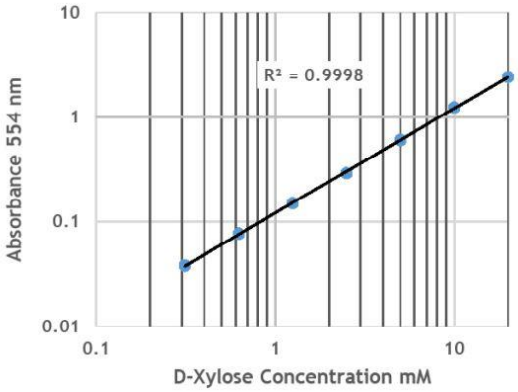
4. The D-Xylose levels in samples can be calculated using regression analysis. If necessary, multiply it by the sample dilution factor to obtain the D-Xylose concentration in the original sample.

   NOTE 1: The D-Xylose concentration can be converted to mg/ml with the following equation.

   $$\text{D-Xylose (mg/ml)} = \frac{150.13}{1000} \text{ ylose (mM)}$$

   NOTE 2: Gut bacteria may metabolize D-Xylose, resulting in a lower D-Xylose uptake in the case of gut bacteria overgrowth (6); further analysis to distinguish bacteria overgrowth from impaired passive carrier-mediated uptake may be required.

Figure 1 – A Typical Standard Curve for the D-Xylose Assay Kit



ASSAY VALIDATION

Table 1 - Reproducibility Data for the D-Xylose Assay Kit

Test	0.6 mM	2.5 mM	10 mM
Intra-Assay CV (%)	5.6	6.7	8.0
Inter-Assay CV (%)	4.6	5.1	6.4
Spike Test* (%)	104%	95%	103%

* Known amounts of D-Xylose were added to samples and then assayed.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s Assay FAQ for more information.

COMPARING TRANSCELLULAR AND PARACELLULAR TESTS (7)

Transcellular Test (D-Xylose)	Paracellular Test (Fluorescent-labeled Dextrans)
This test measures the permeability of small sugar molecules (342 Da or smaller)	This test measures the permeability of large molecules (10,000 Da or larger).
Repair mechanisms can repair small openings in tight junctions within hours.	Large openings in tight junctions (which are associated with structural damage to tight-junction proteins) cannot be repaired within hours.
The intestinal permeability to small sugar molecules does not necessarily correlate with the uptake of much larger dietary antigens and bacterial toxins, such as LPS.	The intestinal permeability to large molecules correlates with digestion-resistant fragments of food antigens and bacterial toxins, such as LPS.
Measuring permeability to small sugar molecules does not correlate with gut dysbiosis, endotoxin release, microbial translocation, and activation of the mucosal immune system.	Measuring permeability to large molecules such as LPS does correlate with gut dysbiosis, microbial translocation, and immune activation.

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