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Product No: **S-7500**
Liquid Stable Conjugate
Approximate Dynamic Range
Dextran 2 mDa 0.03 -10 µg/mL

Dextran ELISA Kit

INTENDED USE: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT INTENDED FOR CLINICAL OR DIAGNOSTIC USE.

Kit includes:

Coated 96-well plate
Detector -Enzyme Conjugate Solution
TMB Solution
Stop Solution
Wash Concentrate 10X, (dilute 1 part plus 9 parts water)
Dextran 2,000,000 MW Standard (10,000 µg/mL)
Blank blue 96 well plate for sample preparation (single use only)
Plate Sealer

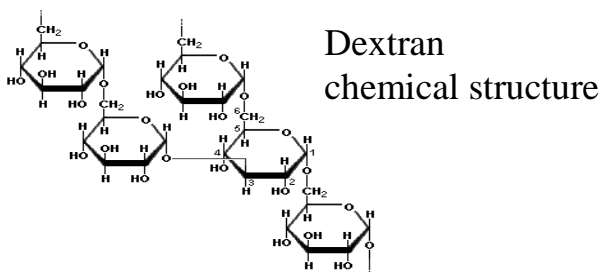
Researcher must provide:

Pipettes (8 Channel Multipipettor is recommended)
Absorbance microplate reader
Reagent Grade Water

Storage and Stability

Kit can be stored unopened at 4°C for up to six months. The Detector-Enzyme Conjugate Solution and the TMB solution should be protected from light.

Background



Dextran is a complex, branched glucan polysaccharide made of many glucose molecules composed of chains of varying lengths. The straight chain consists of α -1,6 glycosidic linkages between glucose molecules, while branches begin from α -1,4 linkages (and in some cases, α -1,2 and α -1,3 linkages as well). Dextran is synthesized from sucrose by certain lactic-acid bacteria, the best known being *Leuconostoc mesenteroides* and *Streptococcus mutans*. It is used medicinally as an antithrombotic (anti-platelet), to reduce blood viscosity and as a volume expander in anemia. It is used as a masking agent for some doping agents utilized in athletic performance enhancement. Dextran is damaging to the sugar industry for several reasons and should be minimized. It inhibits sucrose crystal formation which reduces product yield. It coats the surfaces it touches with a hard precipitate that can harm machinery for sugar producers and their customers. Dextran can cause product perception problems in food, beverages and liqueur.

The Dextran ELISA product number S-7500 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of Dextran levels in fluids such as buffer or diluted juice (or syrup or molasses) from sugar cane or sugar beets. This assay measures Dextran directly using a Dextran antibody which has been conjugated to HRP.

The Dextran ELISA is a competitive assay in which the colorimetric signal is inversely proportional to the amount of Dextran present in the sample. Samples to be assayed are first mixed with the detector-enzyme conjugate in the wells of the coated plate. Dextran in the sample competes with Dextran bound to the plate for binding of the detector-enzyme conjugate. The bound peroxidase is reacted with tetramethylbenzidine (TMB) to quantitate the assay. The concentration of Dextran in the sample is determined using a standard curve of known amounts of Dextran. Parts per million and micrograms per mL are interchangeable. (1 PPM = 1 µg/mL)

Reagent Preparation

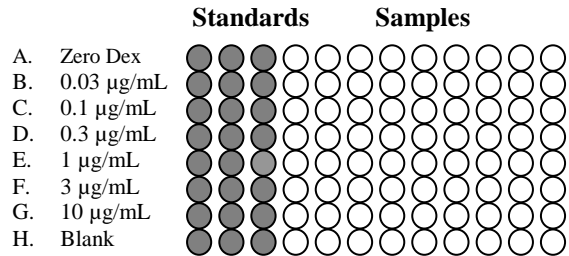
1X Wash Buffer: Make a 1:10 dilution of 10X Wash Buffer in distilled or deionized water

Sample Dilutions:

Sugar Industry: Raw and refined sugar should be prepared at 1%, (1.0 g/100mL) in purified water. Juice and syrup samples may be viscous or may have very high dextran levels and should be diluted with purified water prior to assay. Juice samples should be diluted at 100X and syrup samples at 200X.

Dextran Standards: Make dilutions of the included 10,000 µg/mL Dextran stock, use reagent grade water or 1% sucrose to obtain standards of 0.03, 0.1, 0.3, 1, 3, and 10µg/mL. To prepare standards from the included vial of 10,000µg/mL Dextran, first prepare a stock solution of 100 µg/mL Dextran by adding 0.030 mL of 10,000 µg/mL Dextran to a tube containing 2.97 mL of diluent. Mix well. Then add the appropriate amount of standard diluent and stock solution to each of 7 tubes as outlined in the table below. Mix each tube well. If you are performing less than a full plate, the diluted standards may be stored at 4° C and re-used in your next assay.

Standard	Diluent	Stock
10.00 µg/mL	2.700 mL	0.300 mL of 100 µg/mL
3.00 µg/mL	2.910 mL	0.090 mL of 100 µg/mL
1.00 µg/mL	2.970 mL	0.030 mL of 100 µg/mL
0.30 µg/mL	2.700 mL	0.300 mL of 3 µg/mL
0.10 µg/mL	2.900 mL	0.100 mL of 3 µg/mL
0.03 µg/mL	2.970 mL	0.030mL of 3 µg/mL
0 µg/mL	3.000 mL	0.000 mL of 3 µg/mL



Dextran ELISA

Assay Procedure

1. Set up the Dextran ELISA plate as illustrated above. We suggest the Dextran standard dilution series be run in triplicate for best results. Add **50µl** Standards and samples into corresponding wells. Add **50µl** of Detector-Enzyme conjugate to all wells except the Blank wells. Mix well. Cover plate and incubate for 30 minutes at room temperature. A rotator is highly recommended if available, as constant mixing significantly improves precision.
2. Discard the solution and wash the wells four times with 300 µL per well of 1X Wash Buffer. An automated plate washer is recommended if available. After washing, immediately proceed to the next step. Do not delay in removing wash buffer from the wells. Do not allow the plate to dry.
3. Add **100 µL** of TMB to all wells. Incubate the plate in the dark at room temperature for 10 - 60 minutes waiting for the zero Dextran wells to develop to a medium to dark blue color. Watch for color development and DO NOT overdevelop.
4. Add **50 µL** Stop Solution which will change the color from blue to yellow.
5. Immediately measure the absorbance of each well at 450 nm in an absorbance microplate reader.
6. Calculate the binding percentage for each sample using the formula:

$$[A_{450}(\text{Sample}) - A_{450}(\text{Blank})] / [A_{450}(\text{Zero Dextran}) - A_{450}(\text{Blank})] \times 100 = \% \text{ Binding}$$

Using linear or nonlinear regression, plot a standard curve of percent binding versus concentration of Dextran standards. We usually use a quadratic fit. Determine Dextran levels of unknowns by comparing their percentage binding relative to the standard curve. Dextran can be estimated by comparing the values from the wells containing unknowns to the values in the standard curve.

7. If samples were diluted, adjust results multiplying by the dilution factor.

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