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User's Manual

Human EHEC STX2B ELISA Matched Antibody Pair

REF **DEIABL23**

Σ **15 plates**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B ELISA Pair Set is for the quantitative determination of Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B.

This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

General Description

E. Coli STX2B is a subunit of Stx2. Stx2, together with Stx1, formed a family of related toxins which are known as shiga toxins. Shiga toxins are mainly produced by the bacteria S. dysenteriae and the Shigatoxigenic group of Escherichia coli, which includes serotypes O157:H7, O104:H4, and other enterohemorrhagic E. coli (EHEC). A total of 3222 outbreak cases (including 39 deaths) have been reported in northern Germany in May through June 2011. The outbreak strain was typed as an enteroaggregative Shiga-toxin–producing E. coli O104:H4, producing extended-spectrum beta-lactamase. The toxin has two subunits—A and B. E. Coli STX2B is the B subunit. It is a pentamer that binds to specific glycolipids on the host cell, specifically globotriaosylceramide. Following this, the A subunit is internalised and cleaved into two parts. Stx2 has been found to be approximately 400 times more toxic (as quantified by LD50 in mice) than Stx-1. The Stx1 and Stx2 B subunits form a pentameric structure that binds to globotriaosylceramidereceptors on eukaryotic cells and promotes endocytosis.

Principles of Testing

The CD ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B coated on a 96-well plate. Standards and samples are added to the wells, and any Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

Reagents And Materials Provided

Bring all reagents to room temperature before use.

Capture Antibody: 1 mg/mL of mouse anti-Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating.

Detection Antibody: 0.2 mg/mL of mouse anti-Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 0.5 µg/mL in dilution buffer before use.

Standard: Each vial contains 50 ng of recombinant Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B. Reconstitute with 1 mL dilution buffer. After reconstitution, store at -20°C to -80°C in a manual

defrost freezer. A seven-point standard curve using 2-fold serial dilutions in dilution buffer, and a high standard of 2200 pg/mL is recommended.

Materials Required But Not Supplied

PBS: 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4, 0.2 µm filtered

TBS: 20 mM Tris, 150 mM NaCl, pH 7.4

Wash Buffer: 0.05% Tween20 in TBS, pH 7.2-7.4

Blocking Buffer: 2% BSA in Wash Buffer

Sample dilution buffer: 0.1% BSA in wash buffer, pH 7.2-7.4, 0.2 µm filtered

Detection antibody dilution buffer: 0.5% BSA in wash buffer, pH 7.2-7.4, 0.2 µm filtered

Substrate Solution: To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution: 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer: 0.05M Na_2HPO_4 and 0.025M citric acid; adjust pH to 5.5

Substrate working solution: For each plate dilute 250 µl substrate stock solution in 25 ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2, mix it well

Stop Solution: 2 N H_2SO_4

Storage

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Store at 4°C and protect it from prolonged exposure to light for up to 6 months from date of receipt. Do not freeze!

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80°C for up to 1 month. Avoid repeated freezethaw cycles.

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (if substrate solution is not as requested, the incubation time should be optimized). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

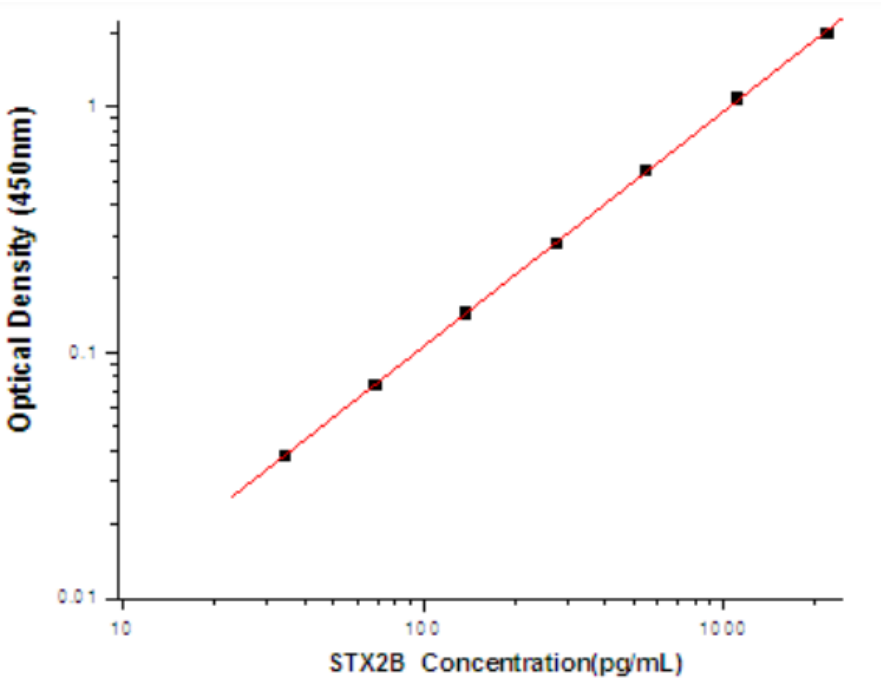
Calculation

1. Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
2. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
34.38	0.038
68.75	0.074
137.5	0.145
275	0.278
550	0.550
1100	1.077
2200	1.995



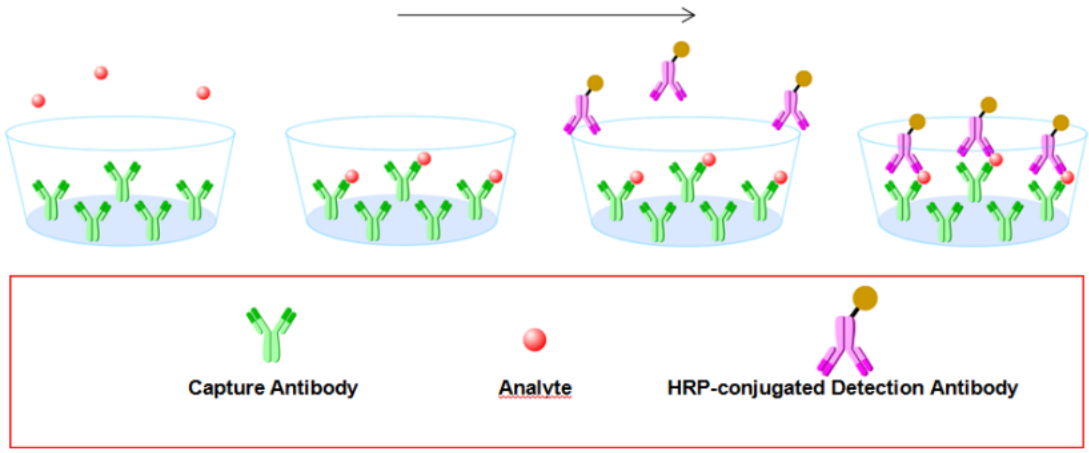
Sensitivity

The minimum detectable dose of Enterohemorrhagic E. coli (EHEC) STX2B / Shiga toxin II subunit B was determined to be approximately **34.38 pg/ml**.

This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

Precautions

The Stop Solution suggested for use with this Pair Set is an acid solution. Wear eye, hand, face, and clothing protection when using this material.



Trouble Shooting

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples