

Human ROBO4 ELISA Kit

Sandwich ELISA

Catalog EL-213-1815204

INTRODUCTION

ROBO4 is a member of the Roundabout receptor family expressed almost exclusively on vascular endothelial cells. It binds Slit ligands, most notably SLIT2, and this interaction restrains endothelial cell migration while helping to organize new blood vessel growth. A separate, largely Slit-independent action of ROBO4 helps hold endothelial cell-cell junctions together, preserving the integrity of the vascular barrier and limiting vessel leakiness. Altered ROBO4 signaling has been linked to bicuspid aortic valve disease, and shifts in its circulating levels have been explored as indicators of vascular stress in conditions such as pre-eclampsia.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format in which the microtiter plate wells are pre-coated with a capture antibody specific to Human ROBO4. Standards or samples are added to the appropriate wells together with a biotin-conjugated detection antibody that also recognizes Human ROBO4. Avidin conjugated to horseradish peroxidase (HRP) is then added to each well and allowed to incubate. Upon addition of TMB substrate solution, a colorimetric signal develops exclusively in wells where Human ROBO4, the biotin-conjugated detection antibody, and avidin-HRP are all present. The enzymatic reaction is stopped by the addition of a sulphuric acid stop solution, and absorbance is measured at 450 nm ($\pm$ 10 nm). Human ROBO4 concentrations in the samples are determined by comparing sample optical density values against a standard curve.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard (Lyophilized)	2 vials
Biotinylated Antibody (100×)	120 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 mL
Biotinylated Antibody Diluent	12 mL
HRP Diluent	12 mL
Wash Buffer (25×)	20 mL
TMB Substrate Solution	10 mL
Stop Reagent	6 mL
Plate Covers	2 pieces
<i>Do not mix or substitute reagents with those from other lots.</i>	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective

clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 10 down to 0.16 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (μL)	Cal. Diluent (μL)	Final Vol (μL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	10
Std 2	Std 1	500	500	1000	5
Std 3	Std 2	500	500	1000	2.5
Std 4	Std 3	500	500	1000	1.25
Std 5	Std 4	500	500	1000	0.63
Std 6	Std 5	500	500	1000	0.32
Std 7	Std 6	500	500	1000	0.16
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 100 μL of Standard Working Buffer (gradually diluted according to the instructions) or 100 μL of sample to each well, and incubate at 37°C for 80 minutes.
2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.
3. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.
4. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.
5. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 0.05 ng/mL

Recovery: Serum (n = 5): 80-92% (avg 86%); EDTA plasma (n = 5): 78-95% (avg 86%); Heparin plasma (n = 5): 80-96% (avg 88%)

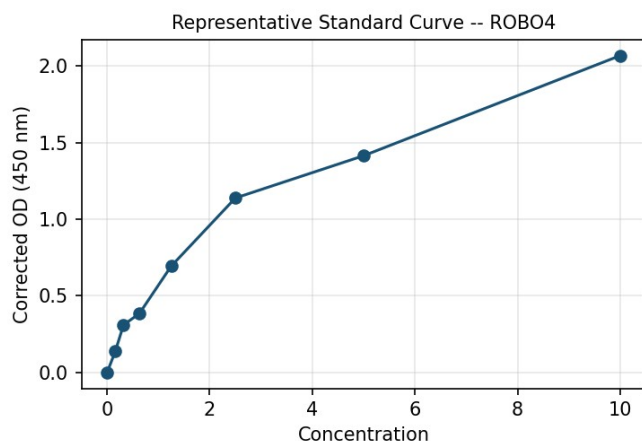
Specificity: This assay has high sensitivity and excellent specificity for detection of Human ROBO4. No significant cross-reactivity or interference between Human ROBO4 and analogues was observed.



This kit employs a sandwich enzyme immunoassay technique to quantify Human ROBO4 in biological samples.

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SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.