This kit employs a sandwich enzyme immunoassay technique to quantify Human ROR1 in biological samples.

Human ROR1 ELISA Kit
Sandwich ELISA
Catalog EL-213-18151801

INTRODUCTION

ROR1 encodes a type I transmembrane glycoprotein whose extracellular region combines kringle, frizzled-related, cysteine-rich, and immunoglobulin-like domains, giving it the overall architecture of a receptor tyrosine kinase, yet its intracellular kinase domain is catalytically dead and does not appear to function as a true enzyme in cells. Instead, ROR1 works as a receptor for the non-canonical Wnt ligand WNT5A, triggering NF-kB signaling along with planar-cell-polarity and calcium-dependent pathways that influence cell division, migration, and chemotaxis, while apparently also dampening signaling from the canonical ligand WNT3A. The receptor is abundant during early embryonic development, including a specific requirement for spiral ganglion neurons to properly innervate auditory hair cells in the inner ear, but its expression falls to very low levels in most healthy adult tissues. That embryonic pattern re-emerges in a wide range of malignancies, including chronic lymphocytic leukemia, mantle cell lymphoma, and cancers of the ovary, breast, prostate, lung, and colon, where ROR1 partners with IGFBP5 and ERBB2 to activate CREB-driven survival signaling and support tumor cell growth.

PRINCIPLE OF THE ASSAY

This kit utilizes a sandwich enzyme immunoassay format. The microtiter plate wells are pre-coated with a capture antibody specific to Human ROR1, which binds the target analyte present in standards or samples added during the first incubation step. A biotin-conjugated detection antibody, also specific to Human ROR1, is then introduced and binds a second epitope on the captured analyte. Avidin conjugated to horseradish peroxidase (HRP) is subsequently added to each well, linking to the biotin-conjugated detection antibody. Upon addition of TMB substrate solution, a colorimetric signal develops only in wells where Human ROR1, the biotin-conjugated antibody, and avidin-HRP are all present. The enzymatic reaction is terminated by the addition of a sulphuric acid stop solution, and absorbance is measured spectrophotometrically at 450 nm (± 10 nm). Sample concentrations are determined by comparing measured 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

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

Do not mix or substitute reagents with those from other lots.

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

amount of reagents. Spin tubes on pulse setting prior to opening.

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: 0.057 ng/mL

Recovery: Serum (n = 5): 78-93% (avg 85%); EDTA plasma (n = 5): 85-97% (avg 91%); Heparin plasma (n = 5): 87-99% (avg 93%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human ROR1. No significant cross-reactivity or interference between Human ROR1 and analogues was observed.

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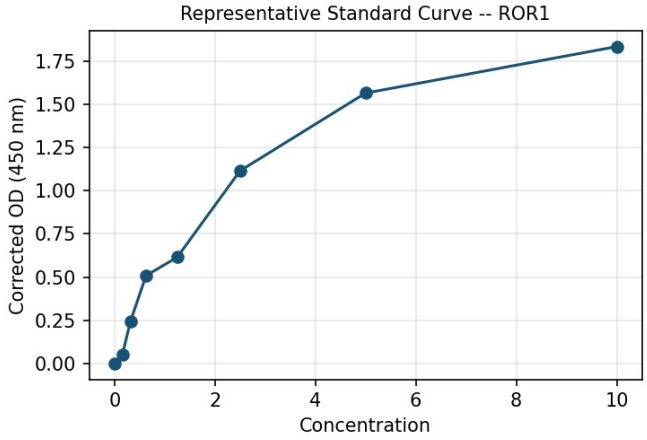
SAMPLE STANDARD CURVE



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.