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

Human TIGIT ELISA Kit
Sandwich ELISA
Catalog EL-213-20

INTRODUCTION

TIGIT is an inhibitory immune checkpoint receptor found on activated and memory T cells, regulatory T cells, and NK cells, built from a single IgV extracellular domain, a transmembrane segment, and a cytoplasmic tail carrying an ITIM motif. Its main ligand, CD155 (PVR), is displayed at high levels on dendritic cells, fibroblasts, and endothelial cells, while related proteins CD112 and CD113 bind with lower affinity; engagement of any of these ligands triggers phosphorylation of the TIGIT tail and recruitment of adaptor and phosphatase proteins that dampen PI3K, MAPK, and NF-kB signaling inside the lymphocyte. The functional outcome is a shift toward an anti-inflammatory cytokine profile, with reduced IL-12 and increased IL-10 output, along with promotion of tolerogenic dendritic cell maturation and progressive functional exhaustion of chronically stimulated T cells, including tumor-infiltrating lymphocytes. Because this same pathway also helps coordinate follicular helper T cell interactions with dendritic cells during antibody responses, TIGIT sits at the intersection of tumor immune evasion and normal regulation of T-cell-dependent B-cell help, making it an actively pursued checkpoint-blockade target.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format to detect and quantify Human TIGIT. Microtiter plate wells are pre-coated with a TIGIT-specific capture antibody that immobilizes the target antigen present in standards or samples added to each well. A biotin-conjugated detection antibody, also specific to Human TIGIT, is then introduced and binds a second epitope on the captured antigen. Avidin conjugated to horseradish peroxidase (HRP) is subsequently added and binds the biotin label, completing the detection complex. Upon addition of TMB substrate solution, a colorimetric signal develops only in wells containing Human TIGIT, the biotin-conjugated detection antibody, and the avidin-HRP conjugate. The enzymatic reaction is stopped by addition of a sulphuric acid stop solution, and the resulting color change is measured spectrophotometrically at 450 nm (± 10 nm). Human TIGIT concentrations in the samples are then determined by comparing sample optical density values against a standard curve.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO

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

NOT USE past kit expiration date. Some vials contain a small 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 20 down to 0.32 (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	20
Std 2	Std 1	500	500	1000	10
Std 3	Std 2	500	500	1000	5
Std 4	Std 3	500	500	1000	2.5
Std 5	Std 4	500	500	1000	1.25
Std 6	Std 5	500	500	1000	0.63
Std 7	Std 6	500	500	1000	0.32
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: 0.135 ng/mL

Recovery: Serum (n = 5): 95-108% (avg 101%); EDTA plasma (n = 5): 83-97% (avg 90%); Heparin plasma (n = 5): 80-97% (avg 88%)

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

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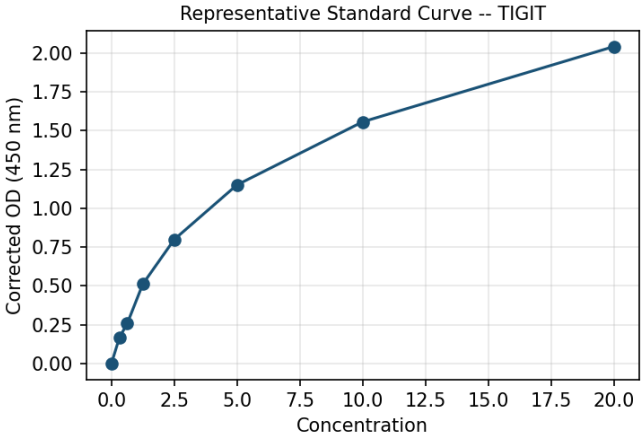
SAMPLE STANDARD CURVE



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.