**Human TCN ELISA Kit**

**CATALOG NO:** IRTCNT **LOT NO:** Sample

INTENDED USE

The Innovative Research Human TCN2 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human TCN2 with a 96-well strip plate that is pre-coated with antibody specific for TCN2. The detection antibody is a biotinylated antibody specific for TCN2. The capture antibody is a monoclonal antibody from mouse and the detection antibody is a biotinylated polyclonal antibody from goat. The kit includes Human TCN2 protein as standards.

BACKGROUND

Repeat steps a-b 2 additional times.  
  
Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.  
  
Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).  
  
Wash the plate 5 times with the 1x wash buffer:  
  
Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.  
  
Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).  
  
Repeat steps a-b 4 additional times.  
  
Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.  
  
Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or  
  
15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)  
  
Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.  
  
Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.  
  
Assay Protocol Notes  
  
Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.  
  
Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.

PRINCIPLE OF THE ASSAY

The Innovative Research Human TCN2 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human TCN2 with a 96-well strip plate that is pre-coated with antibody specific for TCN2. The detection antibody is a biotinylated antibody specific for TCN2. The capture antibody is a monoclonal antibody from mouse and the detection antibody is a biotinylated polyclonal antibody from goat. The kit includes Human TCN2 protein as standards.  
  
To measure Human TCN2, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human TCN2 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human TCN2 in the sample. For more information on assay principle, protocols, and troubleshooting tips, see Innovative Research's ELISA Resource Center at https://www.bosterbio.com/elisa-technical-resource-center.  
  
Overview  
  
\*The sensitivity or the minimum detectable dose (MDD) is the lower limit of the target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.  
  
Technical Details  
  
Preparations Before Assay  
  
Please read the following instructions before starting the experiment.  
  
Read this manual in its entirety in order to minimize the chance of error.  
  
Confirm that you have the appropriate non-supplied equipment available.  
  
Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.  
  
Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.  
  
When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.  
  
If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).  
  
To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.  
  
Before using the kit, spin tubes to bring down all components to the bottom of the tubes.  
  
Don’t let the 96-well plate dry out since this will inactivate active components on the plate.  
  
Don’t reuse tips and tubes to avoid cross-contamination.

SPECIFICATION

REAGENTS

MATERIALS REQUIRED BUT NOT PROVIDED

Microplate reader capable of reading absorbance at 450 nm. Incubator.  
  
Automated plate washer (optional)  
  
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for a large numbers of samples.  
  
Deionized or distilled water. 500 ml graduated cylinders. Test tubes for dilution.  
  
Human TCN2 ELISA Standard Curve Example  
  
The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.  
  
Human TCN2 ELISA Kit Standard Curve A standard curve is provided for demonstration only. A standard curve  
  
should be generated for each set of samples assayed.  
  
Intra/Inter-Assay Variability  
  
Innovative Research spends great efforts in documenting lot-to-lot variability and ensuring our assay kits produce robust data that are reproducible.  
  
Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.  
  
Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.  
  
Reproducibility  
  
We ensure reproducibility by testing three samples with differing concentrations of TCN2 in ELISA kits from four different production batches/lots.  
  
\*number of samples for each test n=16.  
  
Preparation Before The Experiment  
  
Dilution of Human TCN2 Standard  
  
Number tubes 1-8. Final Concentrations to be Tube # 1: 10,000.00 pg/ml, # 2: 5,000.00 pg/ml, # 3: 2,500.00  
  
pg/ml, # 4: 1,250.00 pg/ml,  
  
# 5: 625.00 pg/ml, # 6: 312.50 pg/ml, # 7: 156.25 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).  
  
For standard #1, add 1000 µl of undiluted standard stock solution to tube #1.  
  
Add 300 µl of sample diluent to tubes # 2-7.  
  
To generate standard # 2, add 300 µl of standard # 1 from tube # 1 to tube # 2 for a final volume of 600 µl. Mix thoroughly.  
  
To generate standard # 3, add 300 µl of standard # 2 from tube # 2 to tube # 3 for a final volume of 600 µl. Mix  
  
thoroughly.  
  
Continue the serial dilution for tube # 4-7.

TYPICAL DATA

TYPICAL STANDARD CURVE

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

|  |  |
| --- | --- |
| **Concentration** | **OD Value** |
| 0.0 | 0.129 |
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INTRA/INTER ASSAY VARIABILITY

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter- assay precision.

REPRODUCIBILITY

\*number of samples for each test n=16.

PROCEDURAL NOTES

REAGENT PREPARATION AND STORAGE

DILUTION OF STANDARD

1. Label 7 tubes, one for each standard: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml.  
 2. Pipette 300 µl of the Sample Diluent into each tube.  
 3. Pipette 300 µl of the reconstituted standard into the first tube and mix to create the 4000 pg/ml standard.  
 4. Pipette 300 µl from the 4000 pg/ml tube into the second tube and mix to create the 2000 pg/ml standard.  
 5. Continue this process for the remaining tubes.  
 6. The Sample Diluent serves as the zero standard (0 pg/ml).

SAMPLE COLLECTION & STORAGE

Innovative Research recommends that samples are used immediately upon preparation.  
  
Avoid repeated freeze/thaw cycles for all samples.  
  
In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.  
  
Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.  
  
Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.  
  
Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.

ASSAY PROCEDURE

{% for step in assay\_protocol %}

{{ step }}

{% endfor %}

DATA ANALYSIS

Subtract the average zero standard O.D. reading. It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor. Background on TCN2  
  
TCN2 (Transcobalamin II; also TC-2 or TC) is a 42-44 kDa, monomeric, secreted member of the eukaryotic cobalamin transport family of molecules. This gene is mapped to 22q12.2. This gene encodes a member of the vitamin B12-binding protein family. This family of proteins, alternatively referred to as R binders, is expressed in various tissues and secretions. This plasma protein binds cobalamin and mediates the transport of cobalamin into cells. This protein and other mammalian cobalamin-binding proteins, such as transcobalamin I and gastric intrisic factor, may have evolved by duplication of a common ancestral gene. Alternative splicing results in multiple transcript variants. Submit a Product Review to Biocompare.com  
  
Submit a review of this product to Biocompare.com to receive a $20 Amazon.com gift card! Your reviews help your fellow scientists make the right decisions. Thank you for your contribution. Human TCN2/Transcobalamin-2 ELISA Kit ®

DISCLAIMER

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