SOLUBLE TROP2 (HUMAN) ULTRA-SENSITIVITY ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE TROP2 CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**TROP2 (HUMAN) ULTRA-SENSITIVITY ELISA KIT**
Catalog No.	SK00819-26
Lot No.	
Luminescence Reader	Top
Formulation	96 T
Standard range	0.39 – 6.25 pg/mL
Sensitivity	0.1 pg/mL
Sample Volume	100 μL
Sample Type	Serum, Plasma
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application***
Specificity	Human soluble TROP2
Calibration	Human TROP2 recombinant, extracellular domain (HEK293)
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 8%
Storage	2 - 8° C for 6 months, see page 2 for more information
This kit contains sufficient materials to run approximately 35~40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble TROP2 Ultra-Sensitivity Chemiluminescence ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human TROP2 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human TROP2 derived from HEK293 cells animal free cultures and monoclonal antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human TROP2 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human soluble TROP2. The capture antibody can bind to the soluble TROP2 in the standard and samples. After washing the plate of any unbound substances, a HRP conjugated monoclonal antibody against sTROP2 is added to the wells. After the last wash to remove any unbound enzyme, a mixed chemiluminesence substrate solution is added to the wells and luminescence light develops in direct proportion to the amount of sTROP2 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
sCD66C Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against soluble TROP2.	**819-26-01**	**1 plate**
Soluble TROP2 Standard – 1600 pg/vial of recombinant soluble TROP2 in a buffered protein base with preservative; lyophilized.	**819-26-02**	**1 vial**
Detection Antibody HRP Concentrate – 85 µL/vial, 100-fold concentrated of monoclonal antibody HRP conjugated against TROP2 with preservative; lyophilized.	**819-26-03**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB02**	**1 bottle**
Wash Buffer - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Chemiluminesence Substrate Solution A – 4.2 mL of CL substrate solution A.	**CLA**	**1 bottle**
Chemiluminesence Substrate Solution B – 4.2 mL of CL substrate solution B.	**CLB**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 6 months. For longer storage up to 10 months, unopened Standard, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Detection Antibody-HRP Conjugate and Chemiluminescence Substrate Solution should be stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate Luminescence Reader capable of top measurement Luminescence Light.
- Microplate shaker (200 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples may require 120 fold dilution. A suggested 20 -fold dilution is 5 µL sample + 95 µL **Dilution Buffer (DB01)**. A suggested 120 - fold dilution is 12.5 µL per well of 20-fold diluted sample + 62.5 µL per well of **Dilution Buffer (DB01)**.

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order Code: 00700-01-25, 25 TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Soluble TROP2 Standard - Reconstitute the soluble TROP2 standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 1600 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 1275 µL of Dilution Buffer into the tube #1 . Pipette 450 µL of Dilution Buffer into the tube #2 to #4. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **6.25 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1600 pg/ml
# 1	5 µl of stock	1275 µl	6.25 pg/ml
# 2	150 µl of 1	450 µl	1.56 pg/ml
# 3	150 µl of 2	450 µl	0.39 pg/ml

Detection Antibody HRP Conjugate- For the 96 tests, freshly Pipette 7.92 mL of **Antibody Diluent Solution (DB02)** into a 15 ml centrifuge tube and transfer 80 µl of 100-fold concentrated stock solution to prepare working solution. For the partial strips test, freshly prepare 700 µL per strip (8-wells) of working solution. Store the 100-fold concentrated stock solution at 2 ~ 8 °C for 10 months.

Working Solution of Chemiluminesence Substrate - For the 96 tests, freshly Pipette 4 mL of **Chemiluminesence Substrate Solution A and 4 mL of Chemiluminesence Substrate Solution B** into a 15 ml centrifuge tube ***(protect from light)***. Mix well to prepare working solution prior to use. That working solution should be used within 10 ~ 20 min. Use 8 channel pipettor to transfer 75 µL per well of the mixed working solution to all assay wells.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 75 µL per well of Dilution Buffer to Blank wells.
3. Add 75 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 75 µL of Detection Antibody HRP conjugate working solution to each well. Cover with plate sealer. Incubate for 1 hour on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
9. Add 75 µL of mixed Substrate Solution to each well. Incubate for 4 minutes on microplate shaker at room temperature. **Protect from light.**
10. Set up the Luminescence Reader to read the top of microplates at 6 minutes .

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble TROP2 (HEK293)	100
Mouse TROP2 (HEK293 derived)	0

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	RLU (CORRECTED)
Blank	0 (748)
0.39	596
1.56	3996
6.25	16990

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 70 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 75 µl Detection Antibody HRP working solution to each well. Incubate 1 hour on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 75 µl mixed Substrate solution to each well. Incubate for 4 min on the plate shaker at RT. **Protect from light**.
↓
Read it by a Luminesence Reader (Top) at 6 min.