RAT TNF-α ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF TNF-α CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	RAT TNF-α ELISA
Catalog No.	SK00109-02
Lot No.	
Formulation	96 T
Standard range	15-2000 pg/ml
Sensitivity	5 pg/ml
Sample Volume	100 µl
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Rat TNF-α
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8 °C

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

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC
2348 WALSH AVE., SUITE C
SANTA CLARA, CA 95051
USA
Tel: 408-982-0300
Fax: 408-982-0301
Email: info@AvisceraBioscience.com
Website: www.AvisceraBioscience.com

INTRODUCTION

Rat TNF-α immunoassay is a solid phase ELISA designed to measure TNF-α in cell culture supernates, serum and plasma. It contains recombinant TNF-α and antibodies raised against this protein. It has been shown to accurately quantify recombinant TNF-α. Results obtained with naturally occurring TNF-α samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural TNF-α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Rat TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for Rat TNF-α is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, Avidin-HRP is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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

_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Rat TNF-α Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against Rat TNF-α.	**109-02-01**	**1 plate**
Rat TNF-α Standard – 2000 pg/vial of recombinant TNF-α in a buffered protein base with preservative; lyophilized.	**109-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against Rat TNF-α with preservative; lyophilized.	**109-02-03**	**1 vial**
Positive Control - one vial of recombinant TNF-α; lyophilized.	**109-02-04**	**1 vial**
Avidin-HRP Conjugate - 50 µl/vial, 250-fold concentrated solution of Avidin conjugated to HRP with preservative.	**AVHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB07**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**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, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. Avidin-HRP Conjugate 250-fold concentrated solution (**protect from light**) and other components may be stored at 2 – 8 °C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 ml and 500 ml graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

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

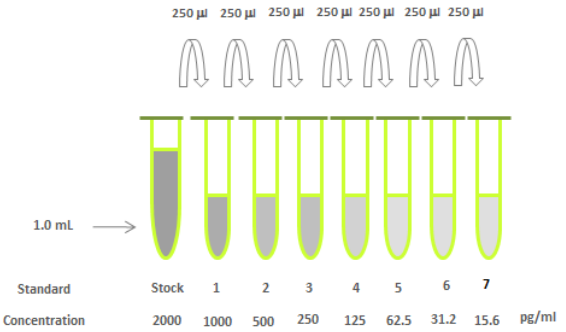
REAGENT PREPARATION

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 50 ml of Wash Buffer Concentrate into deionized or distilled water (450 ml) to prepare 500 ml of 1x Wash Buffer.

TNF-α Standard - Refer to vial label for reconstitution volume. Reconstitute the TNF-α standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 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	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml
# 7	250 µl of 6	250 µl	15.625 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

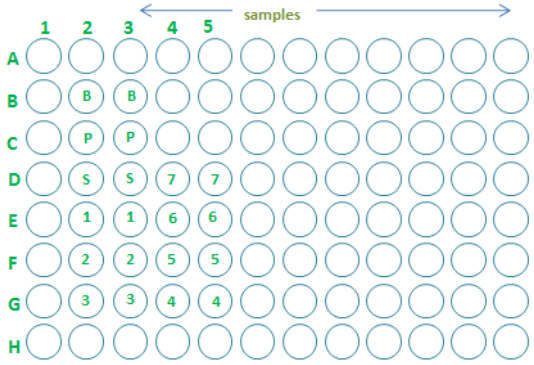
Avidin-HRP Conjugate - Pipette 11.952 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 48 µL of 250-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Avidin-HRP should be used within a few days* ***(protect from light)****.*

Positive Control - Reconstitute the positive control with 0.5 mL of Dilution Buffer to make positive control solution. *Note: Positive control could be used within a few days if stored at -20 °C or -70 °C.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch (P01) with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 µL of **standard solutions in reverse order of serial dilution** (D4, D5 to G4, G5 and G2, G3 to D2, D3), **sample**, or **positive control** (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. 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.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Avidin-HRP Conjugate working solution** to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 1-3 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the standard concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant Rat TNF-α.

SENSITIVITY

The minimum detectable dose (MDD) of TNF-α was 5pg/ml.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.123)
7.813 (optional)	0.066
15.625	0.114
31.25	0.132
62.5	0.226
125	0.343
250	0.652
500	1.221
1000	2.041
2000	2.757

- Lot:
- PC:

SPECIFICITY

This assay recognizes both natural and recombinant Rat TNF-α. The factors listed below were prepared at 50 ng/ml in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/ml in a mid-range rh TNF-α control were assayed for interference. No significant cross-reactivity or interference was observed.

Human Recombinant Proteins:
TNF-α, TNF-β

Mouse Recombinant Proteins:
TNF-α

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

↓

Add 100 µl of **standard, samples, positive control** to the well. Incubate 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µl **Detection Antibody working solution** to each well. Incubate 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µl **Avidin-HRP Conjugate working solution** to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

↓

Add 100 µl **Substrate Solution** to each well. Incubate 1-3 min on plate shaker at RT. **Protect from light.**

↓

Add 100 µl Stop Solution to each well. Read 450nm within 15 min.