

Rat TNF- α

ELISA MAX™ Deluxe Sets

Cat. No. **438204** (5 Plates)
438205 (10 Plates)
438206 (20 Plates)



BioLegend's ELISA MAX™ Deluxe Sets contain the components necessary for the quantification of recombinant and natural rat TNF- α in cell culture supernatant, serum, plasma or other biological fluids. The assay is sensitive, accurate, robust and cost-effective.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Rat TNF- α ELISA Capture Antibody (200X)
2. Rat TNF- α ELISA Detection Antibody (200X)
3. Rat TNF- α Standard
4. Avidin-HRP (1,000X)
5. Matrix Diluent A (For serum and plasma samples only)
6. Substrate Solution A
7. Substrate Solution B
8. Coating Buffer A (5X)
9. Assay Diluent A (5X)
10. NUNC Maxisorp™ 96 MicroWell Plates
11. Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol

Introduction

Rat TNF- α (Tumor Necrosis Factor Alpha), also termed Cachectin, Cytotoxic Factor (CF), CTX, Hemorrhagic Factor, Macrophage-Derived Cytotoxic Factor, and Macrophage Cytotoxic Factor (MCF), is a potent multifunctional cytokine which can exert regulatory, inflammatory and cytotoxic effects on a wide range of normal lymphoid and non-lymphoid cells and tumor cells.

Principle of the Test

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme Linked Immunosorbent Assay (ELISA). A rat TNF- α specific Armenian hamster monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and TNF- α binds to the immobilized capture antibody. Next, a biotinylated goat polyclonal anti-rat TNF- α detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB substrate solution, producing a blue color in proportion to the concentration of TNF- α present in the sample. Finally, the stop solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

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Materials to be Provided by the End-User

- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 μ m filtered.
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20.
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H₂SO₄
- Plate Sealers: BioLegend Cat. No. 423601 is recommended.
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 μ L to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent, aliquot into polypropylene vials and store at -70°C. Do not repeatedly freeze/thaw the recombinant protein standard as loss of activity may occur.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. Substrate Solution A and Substrate Solution B are harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Reagent and Sample Preparation

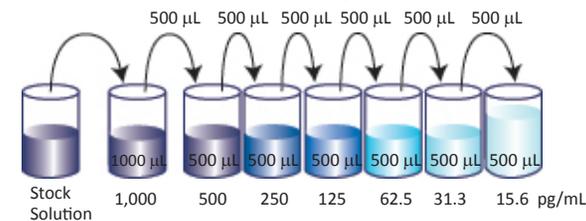
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

1. Dilute 5X Coating Buffer A to 1X with deionized water. For one plate, dilute 2.4 mL 5X Coating Buffer A in 9.6 mL deionized water.
2. Dilute pre-titrated Capture Antibody 1:200 in 1X Coating Buffer A. For one plate, dilute 60 μ L Capture Antibody in 11.94 mL 1X Coating Buffer A.

3. Dilute 5X Assay Diluent A to 1X with 1XPBS. For 50 mL, dilute 10 mL 5X Assay Diluent A in 40 mL 1XPBS.

NOTE: Precipitation of 5X Assay Diluent may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent, it can be filtered to clarify the solution.

4. Lyophilized vials are under vacuum. Reconstitute lyophilized standard with 0.2 mL of 1X Assay Diluent A. Allow the reconstituted standard to sit for 15 minutes at room temperature, then vortex briefly to mix. Aliquot remaining standard into polypropylene vials and store at -70°C.
5. Prior to use, prepare 1 mL of the top standard at a concentration of 1,000 pg/mL from the stock solution in 1X Assay Diluent A (refer to Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol). Perform six two-fold serial dilutions of the 1,000 pg/mL top standard with 1X Assay Diluent A in separate tubes. After diluting, the rat TNF- α standard concentrations are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, and 15.6 pg/mL respectively. 1X Assay Diluent A serves as the zero standard (0 pg/mL).



6. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in 1X Assay Diluent A. For one plate, dilute 60 μ L Detection Antibody in 11.94 mL 1X Assay Diluent A.
7. Dilute Avidin-HRP 1:1,000 in 1X Assay Diluent A. For one plate, dilute 12 μ L Detection Antibody in 11.99 mL 1X Assay Diluent A.
8. TMB Substrate Solution is a mixture of equal volumes of Substrate Solution A and Substrate Solution B. Mix the two components immediately prior to use. For one plate, mix 5.5 mL Substrate Solution A with 5.5 mL of Substrate Solution B in a clean container (solution should be clear and colorless).
9. Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilution is required, samples should be diluted in 1X Assay Diluent A before adding to the wells.

Serum or plasma samples should be tested initially without any dilution. If dilution is required, samples should be diluted in Matrix Diluent A before adding to the wells.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer as described in Reagent Preparation. Add 100 μ L of this Capture Antibody solution to all wells of a 96-well plate provided in this kit. Seal the plate and incubate overnight (16-18 hrs) between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Wash plate 4 times with at least 300 μ L Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 μ L 1X Assay Diluent A per well.

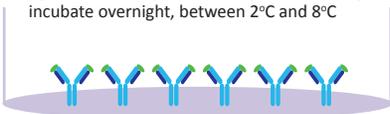
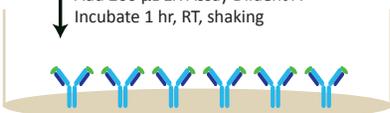
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.
6. While the plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Wash the plate 4 times with Wash Buffer.
8. **For measuring cell culture supernatant:**
Add 50 μ L Assay diluent A to each well. Then add 50 μ L standards to each standard wells or 50 μ L samples to each sample wells. If dilution is required, samples should be diluted in 1X Assay Diluent A before being added to the wells.
- For measuring serum and plasma samples:**
Add 50 μ L Matrix Diluent A to the "standard" wells and 50 μ L Assay Diluent A to the "sample" wells. Then add 50 μ L standards to the "standard" wells or 50 μ L sample to the "sample" wells. If dilution is required, samples should be diluted in Matrix Diluent A before being added to the wells.
9. Seal the plate and incubate at RT for 2 hours with shaking.
10. Wash the plate 4 times with Wash Buffer.
11. Add 100 μ L of diluted Detection Antibody solution to each well, seal the plate and incubate at RT for 1 hour with shaking.
12. Wash the plate 4 times with Wash Buffer.
13. Add 100 μ L of diluted Avidin-HRP solution to each well, seal the plate and incubate at RT for 30 minutes with shaking.
14. Wash the plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100 μ L of freshly mixed TMB Substrate Solution and incubate **in the dark** for 25 minutes*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100 μ L of Stop Solution to each well. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

*Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.

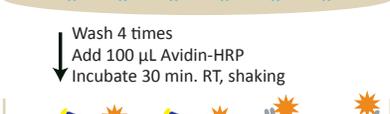
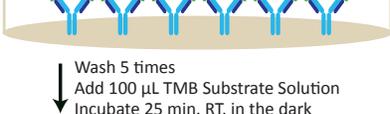
Calculation of Results

Plot the standard curve on log-log axis graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations in the samples, find the absorbance value of the unknown on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the corresponding cytokine concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Assay Procedure Summary

1. Coat plate with 100 μ L diluted Capture Antibody incubate overnight, between 2°C and 8°C

2. Wash 4 times
Add 200 μ L 1X Assay Diluent A
Incubate 1 hr, RT, shaking

3. Wash 4 times
Add 50 μ L Assay Diluent or Matrix A
Add 50 μ L diluted standards or samples
Incubate 2 hrs, RT, shaking

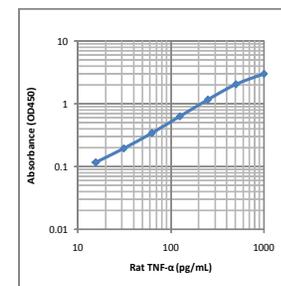
4. Wash 4 times
Add 100 μ L diluted Detection Antibody
Incubate 1 hr, RT, shaking

5. Wash 4 times
Add 100 μ L Avidin-HRP
Incubate 30 min. RT, shaking

6. Wash 5 times
Add 100 μ L TMB Substrate Solution
Incubate 25 min. RT, in the dark

7. Add 100 μ L Stop Solution

8. Read absorbance at 450nm and 570nm

Typical Data

Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics

Sensitivity: The minimum detectable concentration of TNF- α is 2 pg/ml.

Specificity: No cross reactivity was observed when this set was used to analyze multiple human, mouse and rat recombinant proteins other than rat and mouse TNF- α . There is 100% cross reactivity with mouse TNF- α .

Troubleshooting

Refer to the troubleshooting guidelines at BioLegend's website for details (http://www.biolegend.com/elisa_troubleshooting).

BioLegend, Inc.

BioLegend is ISO 9001:2008 and ISO 13485:2003 Certified
9727 Pacific Heights Blvd, San Diego, CA 92121
Tel: 1-858-768-5800, Fax: 1-877-455-9587
www.biolegend.com



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www.biolegend.com/support or
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