



SMC™ Mouse TNF- α

Immunoassay Kit Instructions

Microparticle Assay

Catalog # 03-0137-00

Discovery Immunoassay kit for the quantitative determination of **Mouse Tumor Necrosis Factor α (TNF- α)** in mouse serum

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:



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TABLE OF CONTENTS

INTRODUCTION	2
REAGENTS	3
Reagents Provided	3
Storage Instructions	3
General Supplies Required But Not Provided	4
TECHNICAL HINTS DUE TO HIGH SENSITIVITY	5
SAMPLE INFORMATION	6
PRECAUTIONS	6
ASSAY PREPARATION	7
Microplate Coating	7
Reagent Preparation	7
Sample Preparation	8
Initial Stock Standard Preparation	8
MOUSE TNF- α ASSAY PROCEDURE	9
Standard Curve	9
Target Capture	9
Detection	10
Elution and Plate Transfer	10
Run on Erenna® Immunoassay System	10
APPENDIX A: SMC™ QUICK ASSAY GUIDE	11
TROUBLESHOOTING GUIDE	12
ORDERING INFORMATION	14

INTRODUCTION

The SMC™ Discovery Mouse Tumor Necrosis Factor α (TNF- α) Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure TNF- α in mouse serum samples. The user coats a capture antibody specific for mouse TNF- α onto a 96-well microplate. After blocking the plate, the user pipettes standards and samples into the microplate wells. During incubation, the TNF- α present in the sample binds to the capture antibody on the coated plate. Unbound molecules are washed away during the wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to TNF- α that has been captured onto the plate. Following the final wash step, elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the plate surface, releasing the labeled antibodies. These antibodies are separated during transfer to a final microplate. The plate is loaded into the Erenna® System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of TNF- α present in the sample when captured. The amount of TNF- α in unknown samples is interpolated from a standard curve.

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REAGENTS

The SMC™ Discovery Mouse Tumor Necrosis Factor α (TNF- α) Immunoassay Kit includes all reagents listed in Reagents Provided. Additional reagents and supplies may be required to run this immunoassay, as listed in the section titled General Supplies Required But Not Provided. All reagents supplied are for Research Use Only.

Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	Assay Buffer	With cold pack	2 - 8°C	02-0306-00	1 x 15 mL
2	Mouse TNF- α Coated Antibody	With cold pack	2 - 8°C	02-0890-00	1 x 300 μ L
3	Assay Plate	With cold pack	2 - 8°C	02-0799-00	1 each
4	10X PBS	With cold pack	2 - 8°C	02-0835-00	1 x 1.5 mL
5	Blocking Buffer	With cold pack	2 - 8°C	02-0836-00	1 x 30 mL
6	Standard Diluent	With cold pack	2 - 8°C	02-0864-00	1 x 20 mL
7	Mouse TNF- α Detection Antibody	With cold pack	2 - 8°C	02-0891-00	1 x 100 μ L
8	Mouse TNF- α Standard	On dry ice	$\leq -70^\circ\text{C}$	02-0711-00	1 x 20 μ L
9	10X Wash Buffer (Note: Contains 0.5% Proclin)	With cold pack	2 - 8°C	02-0001-06	2 x 50 mL
10	Buffer C	With cold pack	2 - 8°C	02-0230-01	1 x 3 mL
11	Elution Buffer B	With cold pack	2 - 8°C	02-0211-02	2 x 5 mL

Storage Instructions

- The SMC™ Discovery Mouse Tumor Necrosis Factor α (TNF- α) Immunoassay Kit should be stored at 2 - 8°C. The Standard should be stored at $\leq -70^\circ\text{C}$.
- Discard standards after one use.
- Proper kit performance can only be guaranteed if the materials are stored properly.

REAGENTS (continued)

General Supplies Required But Not Provided

Reagents

1. Erenna® 10X System/Wash Buffer w/ Proclin (1 L bottle) (EMD Millipore PN 02-0111-03)
2. Erenna® 10X Wash Buffer (1 L bottle) (EMD Millipore PN 02-0111-00) if using an automated plate washer
3. Elution Buffer (EMD Millipore PN 02-0002-04) for maintenance
4. De-ionized or distilled water

Washing Options

Automated

- a. Bio-Tek ELx405™ Microplate Washer (EMD Millipore PN 95-0004-05) *or*
- b. Tecan HydroFlex™ microplate washer (EMD Millipore PN 95-0005-02)

Manual

- a. Sphere Mag Plate SBS Footprint (EMD Millipore PN 90-0003-02) *or*
- b. DynaMag™-96 Side Skirted Magnet (Thermo Fisher PN 12027)

Instrumentation / Materials

1. Jitterbug™ Microplate incubator / shaker (EMD Millipore PN 70-0009-00 or equivalent)
2. ALPS™ 50V microplate heat sealer (Thermo Fisher PN AB1443A or equivalent)
3. Centrifuge with plate rotor capable of reaching a speed of 1,100 xg
4. 12-channel pipettes capable of transferring 20 µL - 250 µL
5. 8- or 12-channel pipette capable of transferring 15 µL
6. Rotisserie rotator
7. Microcentrifuge
8. MultiScreen^{HTS} BV 96-Well Filter Plate (EMD Millipore PN MSBVN1210 or equivalent)
9. 96-well V-bottom polypropylene plate, 500 µL (Axygen PN P-96-450V-C)
10. 384-well round bottom polypropylene plate, 120 µL (Thermo Fisher PN 264573)
11. 0.2 µm syringe filter (EMD Millipore PN SLGPR33RS or equivalent)
12. Universal plate cover (Thermo Fisher PN 253623 or equivalent)
13. Sealing tape (Thermo Fisher PN 236366 or equivalent)
14. Heat sealing plate foil (EMD Millipore PN 02-01-0216-00 or equivalent)
15. 12-channel reagent reservoirs for preparing standards
16. 5 mL syringe
17. Microcentrifuge tubes
18. Container capable of holding 300 mL
19. 500 mL graduated cylinder

(Please contact your technical services representative for additional information or assistance selecting required but not provided supplies.)

TECHNICAL HINTS DUE TO HIGH SENSITIVITY

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set-up.

- Wipe down bench and pipettes with 70% isopropanol before use. It is important to allow all reagents to warm to room temperature (20 - 25°C).
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Use filter tips while transferring standard.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation. It is recommended that the standards are prepared as the last step prior to plate setup.
- The detection antibody is light sensitive and must be protected from light at all times.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the wash buffer provided.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- The plates should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate with the pierceable foil seal and store the plate at 2-8°C for up to 48 hrs. Bring to room temperature then centrifuge the plate at 1,100 x g for 5 minutes prior to reading on the Erenna.
- The plate shaker should be set at a speed to provide maximum orbital mixing without splashing liquid on the sealer or outside the wells. For the recommended plate shaker, this would be a setting of 3 - 5.
- For optimal instrument performance, complete a cycle routine (10,000 µL at 1,000 µL/min) followed by a bubble test, and an instrument calibration prior to reading the plate.
- If a clean routine is required, run using three wells of elution buffer (EMD Millipore PN 02-0002-04), one well of 10% bleach and five wells of elution buffer (EMD Millipore PN 02-0002-04). (Note: This elution buffer is not provided and should be ordered separately.)

SAMPLE INFORMATION

- Discovery Assays have a confirmed LLoQ, but no additional verification data. The operator should perform additional verification if so desired.
- The SMC™ Discovery Mouse TNF- α Immunoassay validation data have been compiled using serum.
- Ensure sample is clear of precipitants and other visible particulate matter before testing with the SMC™ Discovery TNF- α Immunoassay.

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.1% sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.
- Proclin-containing solutions and their containers must be disposed of in a safe way and in accordance with local, regional and national regulations.
- The chemical, physical and toxicological properties Proclin 950 at 5% have not been thoroughly investigated. At this concentration, this biocidal preservative is irritating to eyes and skin, and may be detrimental if enough is ingested (quantities above those found in the kit). ProCln 950 is a potential sensitizer by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals. The potential for these adverse health effects is unknown for the highly diluted, small volume of ProCln in this kit, but unlikely if handled appropriately with the requisite good laboratory practices and universal precautions. For full concentration information, please refer to the SDS.

Full Hazardous Label:

Ingredient, Cat #		Full Label	
02-0001-06	10X Wash Buffer		Warning. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

ASSAY PREPARATION

Microplate Coating

1. Prepare 10 mL of 1X PBS by diluting 1 mL of 10X PBS into 9 mL deionized water.
2. Warm the Mouse TNF- α Coating Antibody to room temperature prior to use.
3. Dilute the Coating Antibody 1:100 in 1X PBS (for example, transfer 65 μ L Capture Antibody into 6.435 mL of 1X PBS).
4. Remove the microplate from the package.
5. Pipette 50 μ L of diluted coating antibody into each well of the assay plate. Ensure that the solution has evenly coated the bottom of each well.
6. Seal the plate and incubate without shaking overnight at 4°C.
 - a. NOTE: For consistent results, use this microplate and coating condition. Other configurations have not been tested.

Reagent Preparation

1. Warm the following reagents to room temperature prior to use: Coated Assay Plate, Standard Diluent, Assay Buffer, Blocking Buffer, Elution Buffer B, Buffer C, Detection Antibody and 10X Wash Buffer.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
 - a. Pour 100 mL of the 10X Wash Buffer into a container capable of holding at least 1 L.
 - b. Add 900 mL of deionized water.
 - c. Mix thoroughly by gentle inversion or with a clean, sterile stir bar.
4. Wash the plate 1 time with 250 μ L 1X Wash Buffer. This can be done manually or with an automated plate washer. Ensure that the well is free of residual volume.
5. Block the plate with 200 μ L/well of Blocking Buffer. Incubate with shaking for 1 hour at 25°C on Boekel Scientific, the Jitterbug™ setting 5. The plate can also be blocked overnight at 4°C.

ASSAY PREPARATION (continued)

Sample Preparation

1. Prepare samples by one of the following methods:
 - a. **Serum samples should be diluted at least 1:2 in Standard Diluent prior to measurement.**
 - b. Centrifuge samples at approximately 600 x g for 10 minutes prior to use.
 - c. Samples should be incubated on ice for 10 minutes after centrifugation.
 - d. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

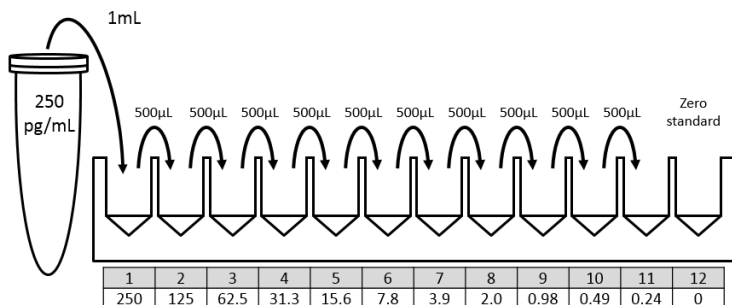
Initial Standard Stock Preparation

1. Quick spin the Mouse TNF- α Standard vial in a microcentrifuge and pipette mix prior to preparing standards. Use care when opening the stock standard vial to prevent loss of materials and contamination of specimens or plates with aerosols.
2. Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the Mouse TNF- α Standard in the vial.
3. To make your Analyte Working Stock, perform the necessary serial dilutions, in Standard Diluent, to achieve the final working concentration of 250 pg/mL in a 1 mL final volume. Ensure that all pipetting steps transfer ≥ 10 μ L of liquid to achieve the best precision.
4. Discard standard after one use.

MOUSE TNF- α ASSAY PROCEDURE

Standard Curve

Prepare the standard curve dilutions in a 12-channel reservoir. Perform 1:2 serial dilutions of the Analyte Working Stock for standards 2 through 11 to achieve a curve from 250 pg/mL to 0.24 pg/mL. Run the standards in triplicate.



1. Add 500 μ L Standard Diluent to wells 2 through 12 of a 12-channel reservoir.
2. Add 1,000 μ L of the 250 pg/mL Analyte Working Stock from standard preparation into well 1.
3. Transfer 500 μ L from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2, stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

Target Capture

1. Wash the plate 1 time with 250 μ L 1X Wash Buffer. This can be performed manually or with an automated plate washer. Ensure that the well is free of residual volume.
2. Pipette 50 μ L per well of Standards or Samples to the Coated Plate.
3. Cover Plate 1 with a plate sealing film.
4. Incubate for 2 hours at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. Approximately 10 minutes prior to the end of Target Capture incubation, prepare the Detection Antibody. Add 65 μ L of Detection Antibody to 6435 μ L of Assay Buffer. Filter the diluted detection antibody using the syringe with a 0.2 μ m filter into a clean tube.
6. When target capture incubation is complete, carefully remove temporary plate cover to avoid splashing.

MOUSE TNF- α ASSAY PROCEDURE (continued)

Detection

1. Wash the plate 3 times with 250 μ L 1X Wash Buffer. This can be performed manually or with an automated plate washer. Ensure that the well is free of residual volume.
2. Add 50 μ L per well of Detection Antibody.
3. Cover plate with plate sealing film.
4. Incubate for 1 hour at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. Carefully remove plate sealing film to avoid splashing.

Elution and Plate Transfer

1. Wash the plate 6 times with 250 μ L 1X Wash Buffer. This can be performed manually or with an automated plate washer. Ensure that the well is free of residual volume.
2. Add 50 μ L Elution Buffer B per well.
3. Cover Assay Plate with a plate cover.
4. Incubate plate for 10 minutes at 25°C on microplate incubator/shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. While the Assay Plate is incubating, add 10 μ L per well of Buffer C to Read Plate (384-well polypropylene plate, Thermo Fisher, PN 264573) using a 12-channel manual P20.
6. Briefly spin the Assay Plate (2 minutes at room temperature, approximately 1,100 x g).
7. Set manual 12 channel pipette to 30 μ L and put 12 tips onto the pipettor. Transfer 30 μ L eluate to Read Plate by rows.
8. Cover Read Plate with heat sealing foil (EMD Millipore PN 02-01-0216-00 or equivalent), according to manufacturer's instructions for the heat sealer.
9. Spin plate for 5 minutes at room temperature at 1,100 x g.

Run on Erenna® Immunoassay System

1. Load completed assay Read Plate onto the Erenna® Immunoassay System.

APPENDIX A: SMC™ Quick Assay Guide

1. Prepare Microplate coating. Add 50 µL of **Diluted Coating Antibody** to each well.
2. Coat sealed plate and incubate for overnight at 4°C on microplate incubator/shaker. **Do Not Shake**



Overnight 4°C

3. Wash **Assay Plate** 1 time with 250 µL **Wash Buffer**.
4. Block plate with 200 µL/well of **Blocking Buffer** for 1 hour at 25°C on microplate incubator/shaker with shaking.
5. Prepare all reagents, standard curve and samples as directed.



1 hour 25°C

6. Wash **Assay Plate** 1 time with 250 µL **Wash Buffer**
7. Add 50 µL of Standard/Diluted Samples to **Assay Plate**.
8. Cover and incubate for 2 hours at 25°C on microplate incubator/shaker.



2 hours 25°C

9. Wash **Assay Plate** 3 times with 250 µL **Wash Buffer**.
10. Add 50 µL of **Detection Reagent** per well.
11. Cover and incubate for 1 hour at 25°C



1 hour 25°C

12. Wash **Assay Plate** 6 times with 250 µL **Wash Buffer**.
13. Add 50 µL of **Elution Buffer B** per well.
14. Cover and incubate for 10 minutes at 25°C



10 minutes 25°C

15. Add 10 µL **Buffer C** per well to **Read Plate**
16. Transfer 30 µL of **Assay Plate** to **Read Plate**.
17. Cover assay Read Plate with pierceable plate seal cover.
18. Cover and centrifuge for 5 minutes at 1,100 x g.



LOAD ON ERENNA® SYSTEM

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including wash and system buffers) are not contaminated.
		Change tips for each dilution of the standard curve.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Instrument needs cleaning	See Technical Guidelines for appropriate Erenna® cleaning protocol.
	Plate was over-incubated	Confirm correct incubation times were followed.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be centrifuged or filtered according to the PI and lab SOPs. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing (~650 - 1000 RPM).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. Should splashing occur on the plate sealer pulse spin plate to remove excess material prior to removing the seal. A new plate seal should be used every time the plate is sealed.

TROUBLESHOOTING GUIDE (continued)

Problem	Probable Cause	Solution
Sample variability is high (continued)	Cross-well contamination (continued)	Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
Published LLoQ was not achieved	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Check plate washer to confirm no beads were lost during washes and that plate contains <2 uL following the post-capture and final aspiration protocols.
		Ensure time from thawing the standard to starting the capture incubation is ≤10 minutes.

ORDERING INFORMATION

To place an order or to obtain additional information about SMC™ products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds

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