Mouse TNF-α Immunospot Assay Kit (ALP)

Cat. No. KITGZ-680 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Immunospot Assay employs a sandwich methodology. A 96-well polyvinylidene difluoride (PVDF) membrane plate, pre-coated with mouse TNF-α-specific monoclonal antibodies, captures mouse TNF-α secreted by activated cells. Following cell removal and washing, biotin-conjugated secondary antibodies targeting TNF-α are added to form antibody-antigen-antibody "sandwich" complexes. Subsequent incubation with streptavidin-alkaline phosphatase (ALP) conjugates enables biotin-mediated binding. Addition of chromogenic substrate culminates in the formation of insoluble spots at the membrane interface, with each spot representing an individual TNF-α-secreting cell. Spot quantification is achieved by automated Immunospot analysis or manual counting using a microscope.
Species	Mouse
Conjugation/Label	ALP
Description	The kit applies for the detection of mouse TNF-α at the cellular level by counting TNF-α-secreting cells. For Research Use Only. Not for use in diagnostic or therapeutic procedures. Please use the kit before the expiration date. Different batches of reagents cannot be mixed. Pack Size: 96 tests.
Form	Kit
Applications	Vaccine development, immunotherapy, drug screening, assessment of infectious and cancer vaccines, immunogenicity evaluation of cell and gene therapies, gene therapy

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	monitoring, infectious disease diagnosis, autoimmune disease research.
Notes	Assay Procedure: 1. Plate Set-up (sterile conditions) Remove the plate from the sealed package and wash 4 times with sterile PBS (200 µl/well). Condition the plate with sterile RPMI-1640 culture media (200 µl/well) containing 10% fetal calf serum (FBS) as used for the cell suspensions. Incubate for at least 30 minutes at room temperature. 2. Incubation of cells in plate (sterile conditions) Empty the plate, then add stimulus followed by the 100 µl cell suspensions to each well. The concentration of cell and stimulus should be adjusted according to the actual situation. The recommended group settings are as follows: a. Positive control: Fewer cells such as 1×10^5 cells/well are needed for polyclonal activators, e.g., LPS (0.1-0.5 µg/ml). b. Negative control: The cell concentration is kept consistent with the experimental wells in the absence of stimulus. c. Background control: No cells, no positive stimulus, add to the medium. d. Experimental group: The recommended cell number per well is 1.5–2.5 × 10^5, and add your own stimulus as required by the experiment (prepare the stimulus using culture medium). Note: It is recommended to set duplicate wells for samples to be tested. Put the plate in a 37°C and 5% CO_2 incubator for 18-48 hours. Do not move the plate during this period and it is recommended to wrap the plate in aluminum foil to avoid evaporation. 3. Detection of spots Note: Use PBS containing 0.5% FBS (PBS-0.5% FBS) for dilution of the Biotin-conjugated antibody and Streptavidin-ALP. The PBS should be filtered (0.2 µm) for optimal results. Avoid the inclusion of Tween or other detergents in the washing and incubation buffers. Remove the cells by emptying the plate and wash 5 times with PBS, 200 µl/well. Note: Soak in the PBS for 1 minute to ensure thorough cleaning. The same requirement applies to all subsequent washing steps. Dilute the detection antibody 1:370 in PBS-0.5% FBS. Add 100 µl/well and incubate for 2 hours at room temperature. Note: Diluted detection antibody can be filtered (0.2 µm) to reduce the risk of unspecific background. Empty the plate and wash 5 times with PBS, 200 µl/well. Dilute the Streptavidin-ALP 1:170 in PBS-0.5% FBS and add 100 µl/well. Incubate for 1 hour at room temperature. Empty the plate and

wash 5 times with PBS, 200 µl/well. Filter the ready-to-use substrate solution (BCIP/NBT-plus) through a 0.45 µm filter and add 100 µl/well. Develop until distinct spots emerge in positive wells (usually 5-30 minutes). Note: Too long the color development time will cause the background color in the experimental well to become darker. Stop color development by washing extensively in tap water. If desirable, remove the underdrain (the soft plastic under the plate) and rinse the underside of the membrane. Leave the plate to dry. Plates should be completely dry before analysis. Inspect and count spots in an Immunospot reader or in a microscope. Note: Do not dry the microplate at a temperature higher than 37°C; this may cause cracking of the membrane filters. For the option to re-analyze later, store plate in the dark at room temperature. Troubleshooting: Deep background color in PVDF membrane: Possible causes: Incomplete plate washing; membrane is wet, not completely dry. Solutions: Increase the number of washing times, ensure each soak lasts 1 minute; allow the PVDF membrane plate to dry completely before reading. Spots appear in negative control wells: Possible causes: Endotoxin or other contaminants in culture medium, serum or DMSO activate cells, leading to additional non-specific spot production; cells are contaminated; low cell viability. Solutions: Use endotoxin-free reagents; pay strict attention to aseptic operation during plate processing and cell incubation; optimize cell isolation and extraction procedures to ensure cell viability and functionality (recommended cell viability >90%). Irregular spot shape or clumping: Possible causes: Cell fragmentation; cell aggregation. Solutions: Optimize cell isolation and extraction procedures to ensure cell viability; mix cell suspension thoroughly to ensure a single-cell state. Few or fuzzy spots in positive control wells: Possible causes: Decreased enzyme activity; chromogenic reagent temperature is low, oxidation phenomenon occurs; low stimulus potency. Solutions: Increase enzyme concentration; equilibrate chromogenic reagent to room temperature before use, do not use reagents that have shown oxidation precipitation; increase stimulus concentration. Uneven spot distribution, edge effect present: Possible causes: Plate was shaken after seeding, causing cell displacement; excessive force when adding stimulus, dispersing cell suspension; plates stacked in incubator. Solutions: Try not to s

	hake the plate after seeding; gently and slowly transfer the plate into the incubator; ad d stimulus before adding cells; place each plate individually on a rack to allow even h eat distribution to each well. Even after parameter adjustment during reading, spots in the well are too dense to be fully counted: Possible causes: Spots fuse together, indiv idual spots cannot be distinguished. Solutions: Reduce the number of cells added to t he well.
Storage	Primary liquid are stable at 2-8°C until expiration date. To be reconstituted, the working fluid is used within the working day and discard. So dilute fresh as needed. Vacuum storage can store at 2-8°C until expiration date.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Reconstitution	Dilute the detection antibody 1:370 in PBS containing 0.5% FBS before use. Dilute the Streptavidin-ALP 1:170 in PBS-0.5% FBS before use. BCIP/NBT-plus Solution A and the Dilution should be mixed together in a 1:250 volume, followed by the addition of Solution B in the same volume as Solution A and mixed gently. The volume ratio of Solution A : Dilution : Solution B is 1:250:1. For example: pipette 40 µL of Solution A into 10 mL of the Dilution buffer, mix thoroughly, then add 40 µL of Solution B and mix gently. Dilute fresh as needed.

GENE INFORMATION

Gene Name	Tnf
Official Symbol	Tnf
Synonyms	DIF; Tnf; TNF-a; TNF-alpha; Tnfa; TNFalpha; Tnfsf1a; TNFSF2; Tnlg1f

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Gene ID	21926
mRNA Refseq	NM_013693
Protein Refseq	NP_038721
UniProt ID	P06804

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