Human IL-2 Immunospot Assay Kit (ALP)

Cat. No. KITGZ-675 **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 human IL-2-specific monoclonal antibodies, captures human IL-2 secreted by activated cells. Following cell removal and washing, biotin-conjugated secondary antibodies targeting IL-2 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 IL-2-secreting cell. Spot quantification is achieved by automated Immunospot analysis or manual counting using a microscope.
Species	Human
Conjugation/Label	ALP
Description	The kit applies for the detection of human IL-2 at the cellular level by counting IL-2-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 seale d package and wash 4 times with sterile PBS (200 µl/well). Condition the plate with st erile 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 fol lowed by the 100 µl cell suspensions to each well. The concentration of cell and stimu lus should be adjusted according to the actual situation. The recommended group sett ings are as follows: a. Positive control: The anti-CD3 mAb, included in the kit, is reco mmended as a positive control at a dilution of 1:125. Add 10 µl of the stimulus per wel l. Fewer cells such as 1.5×10^5 cells/well are needed for this positive control group. b. Negative control: The cell concentration is kept consistent with the experiment al wells in the absence of stimulus. c. Background control: No cells, no positive st imulus, add to the medium. d. Experimental group: The recommended cell num ber per well is $1.5–2.5 \times 10^5$, and add your own stimulus as required by the exper iment (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 i ncubator for 18-48 hours. Do not move the plate during this period and it is reco mmended 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 Bio tin-conjugated antibody and Streptavidin-ALP. The PBS should be filtered (0.2 µ m) for optimal results. Avoid the inclusion of Tween or other detergents in the washin g and incubation buffers. Remove the cells by emptying the plate and wash 5 times wi th 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 Biotin-conj ugated 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 µ

l/well. Dilute the Streptavidin-ALP 1:170 in PBS-0.5% FBS and add 100 µl/well. Incub ate 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, n ot completely dry. Solutions: Increase the number of washing times, ensure each soa k lasts 1 minute; allow the PVDF membrane plate to dry completely before reading. S pots appear in negative control wells: Possible causes: Endotoxin or other contamina nts in culture medium, serum or DMSO activate cells, leading to additional non-specifi c spot production; cells are contaminated; low cell viability. Solutions: Use endotoxin-f ree reagents; pay strict attention to aseptic operation during plate processing and cell incubation; optimize cell isolation and extraction procedures to ensure cell viability an d functionality (recommended cell viability >90%). Irregular spot shape or clumping: P ossible causes: Cell fragmentation; cell aggregation. Solutions: Optimize cell isolation and extraction procedures to ensure cell viability; mix cell suspension thoroughly to e nsure a single-cell state. Few or fuzzy spots in positive control wells: Possible causes: Decreased enzyme activity; chromogenic reagent temperature is low, oxidation pheno menon occurs; low stimulus potency. Solutions: Increase enzyme concentration; equil ibrate chromogenic reagent to room temperature before use, do not use reagents that have shown oxidation precipitation; increase stimulus concentration. Uneven spot dist ribution, edge effect present: Possible causes: Plate was shaken after seeding, causi

	ng cell displacement; excessive force when adding stimulus, dispersing cell suspensi on; plates stacked in incubator. Solutions: Try not to shake the plate after seeding; ge ntly and slowly transfer the plate into the incubator; add stimulus before adding cells; place each plate individually on a rack to allow even heat distribution to each well. Ev en after parameter adjustment during reading, spots in the well are too dense to be ful ly counted: Possible causes: Spots fuse together, individual spots cannot be distingui shed. Solutions: Reduce the number of cells added to the 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 positive control 1:125 in RPMI-1640 culture media containing 10% fetal bovine serum before use. 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	IL2
Official Symbol	IL2

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Synonyms	IL-2; lymphokine; TCGF
Gene ID	3558
mRNA Refseq	NM_000586
Protein Refseq	NP_000577
MIM	147893
UniProt ID	P60568

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