Datasheet for ABIN625147

IL-4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IL-4 (IL4)
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	0.6-200 pg/mL
Minimum Detection Limit:	0.6 pg/mL
Application:	ELISA

Product Details

Purpose:	Mouse IL-4 ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Serum, Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-3 Rb, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 beta, MIP-3 alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1 alpha, TARC, TCA-3, TECK, TIMP-1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM-1, VEGF.
Sensitivity:	< 0.6 pg/mL

Product Details

Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	IL-4 (IL4)
Alternative Name:	IL-4 (IL4 Products)
Background:	Mouse Interleukin-4 (IL-4) is a 20 kDa single chain glycoprotein that is synthesized as a 140 amino acid. IL-4 enhances expression of class II MHC antigens on B-cells. It can promote their capacity to respond to other B-cell stimuli and to present antigens for T-cells. IL-4 inhibits cell activation of NK-cells induced by IL-2. IL-4 stimulates the proliferation of thymocytes with the marker spectrum CD4 (-) CD8 (-), CD4 (+)CD8 (-),CD4 (-)CD8(+). The Mouse IL-4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse IL-4 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse IL-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IL-4 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the

Target Details

	wells and color develops in proportion to the amount of IL-4 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	16189
UniProt:	P07750
Pathways:	JAK-STAT Signaling, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Proton Transport, Activated T Cell Proliferation

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants. Suggested dilution for normal serum/plasma: 2 fold*. * Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 4 µL IL-4

	standard from the vial of Item C (50 ng/mL), into a tube with 996 µL Assay Diluent A or 1x Assay Diluent B to preparea 200 pg/mL stock standard solution. Pipette 450 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 300 µL 300 µL 300 µL 300 µL 300 µL 4 µL standard + 996 µL 300myl 200 80 32 12.8 5.12 2.05 0.82 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 100-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 600-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 25 µL of HRP-Streptavidin concentrate into a tube with 15 ml 1x Assay Diluent B to prepare a 600-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette 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. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step

Application Details

	8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Mouse IL-4 concentration (pg/mL) 0.1 1 10 100 1000 O D =4 50 n m 0.01 0.1 1 10 Assay Diluent B Mouse IL-4 concentration (pg/mL) 0.1 1 10 100 1000 O D =4 50 n m 0.01 0.1 1 10 Sensitivity: The minimum detectable dose of IL-4 is typically less than 0.6 pg/mL. Recovery: Recovery was determined by spiking various levels of mouse IL-4 into mouse serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 95.24 83-102 Plasma 91.43 81-104 Cell culture media 98.54 86-106 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 96 97 98 Range (%) 84-103 84-104 85-104 1:4 Average % of Expected 94 96 97 Range (%) 83-103 84-104 85-105 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Fang, Yao, Li, Wang, Wang, Chen, Zhou, Liao: "The blockage of the Nogo/NgR signal pathway in

microglia alleviates the formation of Aβ plaques and tau phosphorylation in APP/PS1 transgenic mice." in: **Journal of neuroinflammation**, Vol. 13, Issue 1, pp. 56, (2017) (PubMed).

Ahmad, Attia, Bakheet, Zoheir, Ansari, Korashy, Abdel-Hamied, Ashour, Abd-Allah et al.: "Naringin attenuates the development of carrageenan-induced acute lung inflammation through inhibition of NF-?b, STAT3 and pro-inflammatory mediators and enhancement of I?B? and anti-inflammatory ..." in: **Inflammation**, Vol. 38, Issue 2, pp. 846-57, (2015) (PubMed).

Dey, Chaudhuri: "Immunomodulatory activity of Nerium indicum through inhibition of nitric oxide and cyclooxygenase activity and modulation of TH1/T H2 cytokine balance in murine splenic lymphocytes." in: **Cytotechnology**, (2015) (PubMed).

Ghosh, Mukherjee, Choudhury, Gupta, Adhikary, Baral, Chattopadhyay: "Reactive oxygen species in the tumor niche triggers altered activation of macrophages and immunosuppression: Role of fluoxetine." in: **Cellular signalling**, Vol. 27, Issue 7, pp. 1398-412, (2015) (PubMed).

Huang, Ma, Zhu, Chen, Jiang, Zhou, Cen, Pi, Chen: "Total glucosides of peony attenuates experimental autoimmune encephalomyelitis in C57BL/6 mice." in: **Journal of neuroimmunology**, Vol. 284, pp. 67-73, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images

ELISA

Image 1.