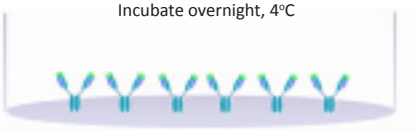
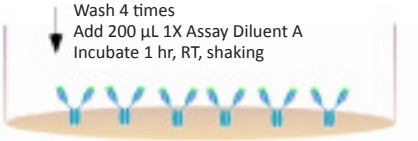
Assay Procedure Summary

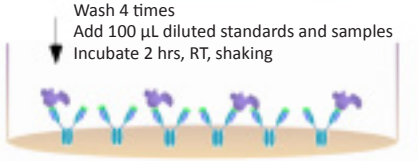
1. Coat plate with 100 µL diluted Capture Antibody
   Incubate overnight, 4°C



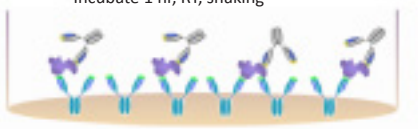
2. Wash 4 times
   Add 200 µL 1X Assay Diluent A
   Incubate 1 hr, RT, shaking



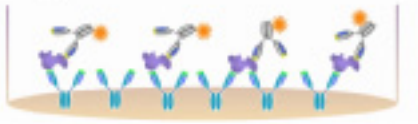
3. Wash 4 times
   Add 100 µL diluted standards and samples
   Incubate 2 hrs, RT, shaking



4. Wash 4 times
   Add 100 µL Detection Antibody
   Incubate 1 hr, RT, shaking



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



6. Wash 5 times
   Add 100 µL Substrate Solution
   Incubate 10-15 min. RT, in the dark

7. Add 100 µL Stop Solution

8. Read absorbance at 450 nm and 570 nm

Performance Characteristics

Sensitivity: The expected minimum detectable concentration of IL-32α for this set is 4 pg/mL.

Specificity: No cross reactivity was observed when this kit was used to analyze 11 human, 8 mouse and 2 rat recombinant cytokines.

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

BioLegend, Inc.
9727 Pacific Heights Blvd, San Diego, CA 92121
Tel: 1-858-768-5800, Fax: 1-858-455-9587
www.biolegend.com

For other technical resources, please visit:
www.biolegend.com/support or
email: **techserv@biolegend.com**

BioLegend
The path to legendary discovery™

Human IL-32α

ELISA MAX™ Deluxe Sets

Cat. No. 433504 (5 Plates)
433505 (10 Plates)

BioLegend's ELISA MAX™ Deluxe Sets contain the components necessary for the accurate quantification of natural and recombinant human IL-32α. These sets are designed for cost-effective and accurate quantification of human IL-32α in cell culture supernatant, serum, plasma or other biological fluids. They are sensitive, accurate, and robust.

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. Human IL-32α ELISA MAX™ Capture Antibody (200X)
2. Human IL-32α ELISA MAX™ Detection Antibody (200X)
3. Human IL-32α Standard
4. Avidin-HRP (1000X)
5. Substrate Solution A
6. Substrate Solution B
7. Coating Buffer A (5X)
8. Assay Diluent A (5X)
9. NUNC Maxisorp™ 96 MicroWell Plates
10. Instruction Sheet
11. Lot-Specific Instruction/ Analysis Certificate

Introduction

Interleukin 32 (IL-32), previously known as NK4, is produced by mitogen-activated lymphocytes, by IFN-γ activated epithelial cells or by IL-12 and IL-18 activated NK cells. Its expression is increased following activation of T cells by mitogens or activation of NK cells by IL-2. IL-32 was shown to activate NF-kB and p38 MAPK in cytokine signal pathways.

Principle of the Test

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A human IL-32α specific monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL-32α binds to the immobilized capture antibody. Next, a biotinylated anti-human IL-32α 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 IL-32α 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

- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Deionized (DI) water
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered.
- Wash Buffer (Phosphate-Buffered Saline (PBS) + 0.05% Tween-20, pH7.4). BioLegend Cat. No. 421601 is recommended.
- Wash bottle or automated microplate washer
- Stop Solution (2N H_2SO_4)
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Sealer
- Absorbent paper

Storage Information

- Store kit components at 4°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent A, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18-25°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/support/#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 Preparation

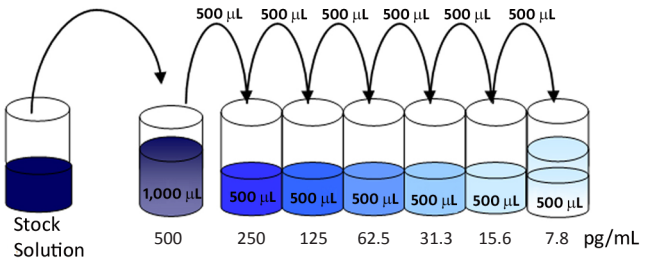
Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

1. Dilute 5X Coating Buffer to 1X with deionized water. For one plate, dilute 2.4 mL 5X Coating Buffer in 9.6 mL deionized water.
2. Dilute pre-titrated Capture Antibody 1:200 in 1X Coating Buffer. For one plate, dilute 60 µL Capture Antibody in 11.94 mL 1X Coating Buffer.
3. Dilute 5X Assay Diluent A to 1X with PBS (pH 7.4). For 50 mL, dilute 10 mL 5X Assay Diluent A in 40 mL PBS.
   NOTE: Precipitation of 5X Assay Diluent A may be observed when stored long term at 4°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.
4. Lyophilized vials are under vacuum pressure. 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 mix gently prior to making dilutions.
5. Prior to use, prepare 1,000 µL of the top standard at a concentration of 500 pg/mL from the stock solution in 1X Assay Diluent A (**refer to Lot-Specific Instruction/Analysis Certificate**).
6. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in 1X Assay Diluent. For one plate, dilute 60 µL Detection Antibody in 11.94 mL 1X Assay Diluent A.
7. Dilute Avidin-HRP 1:1000 in 1X Assay Diluent A. For one plate, dilute 12 µL Avidin-HRP in 11.99 mL 1X Assay Diluent A.
8. TMB Substrate Solution is a mixture of equal volumes of Substrate Solution A with Substrate Solution B. Mix the two components immediately prior to use. For one plate mix 6 mL Substrate Solution A with 6 mL of Substrate Solution B in a clean container (solution should be clear and colorless).

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 µL of this Capture Antibody solution to all wells of a 96-well plate provided in this set. Seal plate and incubate overnight (16-18 hrs) at 4°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 µL 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 µL 1X Assay Diluent A per well.
5. Seal plate and incubate at RT for 1 hour with shaking at 200 rpm on a plate shaker.
6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Prepare 1,000 µL of top standard at 500 pg/mL from stock solution in 1X Assay Diluent A (**refer to Reagent Preparation**). Perform six two-fold serial dilutions of the 500 pg/mL top standard with 1X Assay Diluent A in separate tubes. After diluting, the human IL-32α standard concentrations are 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, and 7.8 pg/mL, respectively. 1X Assay Diluent A serves as the zero standard (0 pg/mL).



8. Wash plate 4 times with Wash Buffer.
9. Add 100 µL/well of standards or samples to the appropriate wells. If dilution is required, samples should be diluted in 1X Assay Diluent A before adding to the wells.
10. Seal plate and incubate at RT for 2 hours with shaking.
11. Wash plate 4 times with Wash Buffer.
12. Add 100 µL of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
13. Wash plate 4 times with Wash Buffer.
14. Add 100 µL of diluted Avidin-HRP solution to each well.
15. Seal plate and incubate at RT for 30 minutes with shaking.
16. Wash 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.
17. Add 100 µL of freshly mixed TMB Substrate Solution and incubate **in the dark** for 10-15 minutes*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
18. Stop reaction by adding 100 µL of Stop Solution to each well. Positive wells should turn from blue to yellow.
19. Read absorbance at 450 nm within 30 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 analyte concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown analyte 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 analyte 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.

Typical Data

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