Tribo™ Mouse KC ELISA Kit (Catalog# TBS3060)

DESCRIPTION

Keratinocyte chemoattractant (KC), also known as CXCL1, SCYB1, or N51 is a member of the CXC or alpha chemokine family. KC is similar in structure and activity to IL-8 and MIP-2. It is produced by melanocytes, epidermal keratinocytes, monocytes, macrophages, mammary epithelial cells, endothelial cells, neutrophils, fibroblasts and hepatocytes. Expression level of this chemokine is normally very low in most tissues however, it can be significantly elevated in many disease states including rheumatoid arthritis, cancer, and experimental autoimmune encephalopathy, post surgery and other inflammatory responses.

The Tribo™ Mouse KC ELISA Kit is designed to detect mouse KC level quantitatively in different tissues including skin, muscle, neural, serum and other biological samples. The plate has been coated with murine KC monoclonal antibody. Any KC present in the sample or standard will be captured. The procedure calls for a biotin-conjugated anti-murine KC antibody to be added along with streptavidin-horseradish peroxidase (HRP) with an accompanying substrate. The reaction exhibits a blue color in direct proportion to the amount of KC in the initial sample. The reaction is terminated by the addition of a stop solution and absorbance is measured at 450nm. The concentration of KC in the samples is calculated opposite to the standard curve.

APPLICATIONS

Direct Assays: quantitative KC in plasma and serum.

KIT CONTENTS

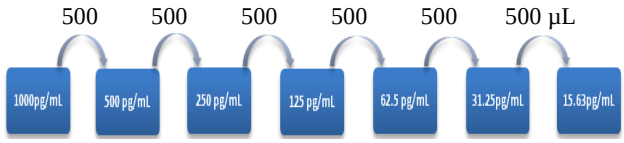
Reagents	Quantity
Precoated 96-well Plate (8x 12 strips)	1 plate
Standard	2x 100 µL
Detection A	1x 100 µL
Detection B	1x 100 µL
Sample Diluent (10x concentrate)	1x 12 ml
TMB Substrate Solution	1x 12 ml
Stop Solution	1x 12 ml
Wash Solution (20x concentrate)	1x 50 ml
Plate Sealers	4

Storage conditions: Store the kit at 4°C and standard at -20°C, respectively.
Shelf life: 12 months after receipt.

PROCEDURES

Reagent Preparation:

1. Bring all reagents to room temperature (18-25˚C) before use. Briefly spin down all small tubes.
2. Wash Buffer: Add 50mL of 20x concentrated Wash Buffer to 950mL distilled water and mix thoroughly.
3. Reagent Diluent: Dilute 10mL of 10x concentrated Sample Diluent with 90mL of deionized water (1:10) before use.
4. Dilute Detection A: Add 100µL of the Detection A into 10mL of the Reagent Diluents for one 96-well plate.
5. Dilute Detection B: Add 100µL of the Detection B into 10mL of the Reagent Diluent for one 96-well plate.
6. Standard: Set up 7 points of KC standard concentration: 1000, 500, 250, 125, 62.5, 31.3 and 15.6 pg/mL as shown in the diagram below.
7. Label 7 eppendoule tubes 1-7. Add 900µL of the Reagent Diluents to the tube #1. Add 500µL of the Reagent Diluents to tubes #2-7.
8. Add 100µL of the KC standard into tube #1 and vortex. This is Standard tube #1 with a concentration of 1000pg/mL.
9. Standards #2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Mix each tube thoroughly before the next transfer. For example, to make Standard #2, remove 500µL of Standard #1 and add it to tube #2 and vortex and so on. The Reagent Diluents serves as the zero standard (0 pg/ml).



Test Procedures

1. Add 100µL of standards, blank or sample into each well of the plate in duplicate manner. Seal the plate with plate sealer and then incubate for 2 hrs at room temperature.
2. Remove sealer and empty wells. Wash the plate 3 times with 200µL of Wash Buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 100µL of the diluted Detection A to each well. Seal the plate and incubate for 2 hrs at room temperature.
4. Repeat the aspiration/washing the plate as in step 2.
5. Add 100µL of the diluted Detection B to each well. Seal the plate and incubate for 20 min at room temperature.
6. Repeat the aspiration/washing the plate as in step 2.
7. Add 100µL of TMB Substrate Solution to each well. Incubate for 20 min at room temperature. Protect from light.
8. Add 50µL of Stop Solution to each well when the highest standard has developed a dark blue color. Gently tap the plate to ensure thoroughly mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

CALCULATION

Average the duplicate readings for each standard, blank and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse KC concentration versus the log of the O.D. and the best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

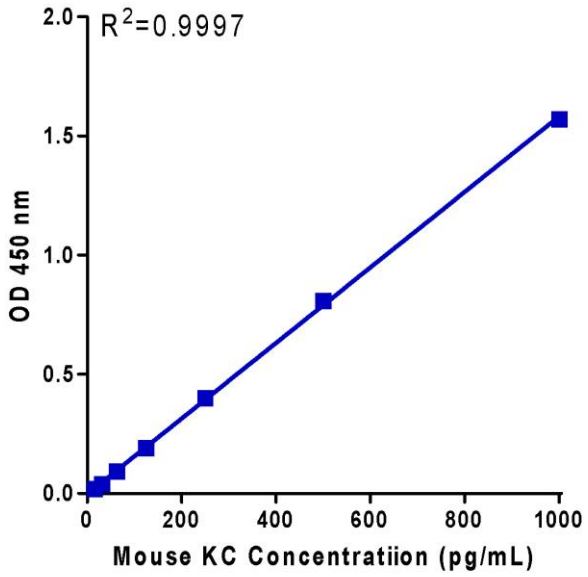
- Microplate reader
- Pipettes and Pipette tips
- Microtubes

MAIN FEATURES

Sensitivity: The minimum detectable dose of mouse KC is less than 8.0 pg/mL typically.

Specificity: This assay recognizes recombinant and natural mouse KC. No cross-reactivity with any of other cytokine is observed.

Standard Curve of KC



REFERENCES

1. Molls RR. et al. Keratinocyte-derived chemokine is an early biomarker of ischemic acute kidney injury. Am J Physiol Renal. 290: 1187-1193 (2006).
2. Armstrong DA. et al. Neutrophil chemoattractant genes KC and MIP-2 are expressed in different cell populations at sites of surgical injury. J Leukoc Biol. 75: 641-648 (1995)

RELATED PRODUCTS:

BSA Standard solution (Catalog# TBS5002)

Protein Cell Lysis Buffer (Catalog# TBS5001)

Protein Assay Kit (Catalog# TBS2005)

Mouse C5a ELISA (Catalog# TBS3001)

Mouse C3a ELISA (Catalog# TBS3020)

Mouse IL-1β ELISA (Catalog# TBS3030)

Mouse IL-6 ELISA (Catalog# TBS3040)

Mouse TNF-α ELISA (Catalog # TBS3050)

Mouse NGF ELISA (Catalog# TBS3070)

Mouse G-CSF ELISA (Catalog# TBS3080)

TMB Substrate System (Catalog# TBS5021)