Mouse KC (IL-8) ELISA kit
(96 Tests)

E22-MC104-96

Mouse KC mouse keratinocyte-derived cytokines (keratinocyte-derived Cytokine, KC), also known as CXCL 1 or N51, were originally identified from fibroblasts as an early gene (Immediate early gene), which encodes a secreted protein of about 8 kDa. According to the protein sequence of the mouse KC, it belongs to the alpha (CXC) chemokine family. In addition to mitogen-activated fibroblasts, KC can also induce expression on peritoneal and lung macrophages, endothelial cells, and vascular smooth muscle cells. It has been shown that glucocorticoids can inhibit the induced expression of KC by mitogens. The cDNA of mouse KC encodes a precursor protein of 96 amino acid residues and the N terminal 19 is cleaved to produce a mature KC protein of 77 amino acid residues. The protein sequence of the mouse KC shares 63% sequence identity with another alpha chemokine, MIP-2. Similar to other alpha chemokines, murine KC is a strong inducer and activator of neutrophils. The biological activity of murine KC and MIP-2 is mediated through the unique murine IL-8 receptor. Mouse IL-8R shares 71% and 68% sequence identity with human IL-8R ß and IL-8R α, respectively. Since no IL-8 homologue has been identified in mice, MIP-2 and KC are thought to be functionally similar to IL-8 as major pro-inflammatory response alpha-chemokines in mice.

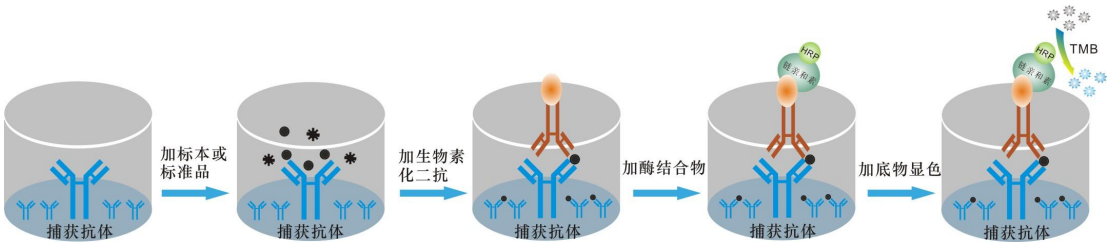
Composition of kit:

name	specifications	Save conditions
Antibodies precoated microplates	8×12 or 8×6*	4℃
Freeze-dried standard	3 / 2 branches * 2 ng / branch	-20℃
Standard & Sample General Dilutions	1 bottle of 20ml / 12ml *	4℃
Concentrated biotinylated antibody	1 Branch *	4℃
Biotinylated antibody dilution solution	1 bottle of 16ml / 10ml *	4℃
Concentrated enzyme conjugates	1 Branch *	4℃ (light avoid)
Enzyme conjugate dilution solution	1 bottle of 16ml / 10ml *	4℃
Concentrate wash solution 20×	1 bottle of 50ml / 25ml *	4℃
Colorogenic substrate (TMB)	1 bottle of 12ml / 6ml *	4℃ (light avoid)
Reaction termination solution	1 bottle of 12ml / 6ml *	4℃
Cover board of gum paper	6 / 3 pieces *	
Product manual	1 piece	

*: [96/48 Tests]

Detection principle:

Double-antibody sandwich ELISA was used in this experiment. The anti-mouse KC (IL-8) mAb is coated on a microplate, and the KC (IL-8) in the specimen and standard binds to the mAb, and the free components are washed away. Biotinylated anti-mouse KC (IL-8) antibody and horseradish peroxidase-labeled avidin were added. Biotin was specifically bound to avidin; the anti-mouse KC (IL-8) antibody was combined to the mouse KC (IL-8) bound to the mAb to form an immune complex, and the free components were washed away. If the chromogenic substrate has KC (IL-8) in the reaction well, horseradish peroxidase will make the colorless chromogenic agent blue and add the termination solution yellow. OD was measured at 450nm, KC (IL-8) concentration versus OD_{450}In a direct ratio between the values, the concentration of KC (IL-8) in the specimen can be obtained by drawing a standard curve.



Schematic representation of the double-antibody sandwich method, ELISA

Specimen collection:

1. The tube for blood collection should be disposable pyrogen-free and endotoxin-free tubes.
2. EDTA is recommended for plasma anticoagulants. Avoid the use of hemolytic, hyperlipid specimens.
3. The specimen should be clear and transparent, and the suspended matter should be removed by centrifugation.
4. If the specimens are not tested in time after collection, please pack them according to the one-time dosage and freeze them in the-20℃ and-70℃ refrigerator to avoid repeated freezing and thawing, and test within 3-6 months.
5. If the concentration of the test substance in your sample is higher than the highest value of the standard, please make an appropriate multiple dilution according to the actual situation (it is recommended to do the pre-experiment to determine the dilution multiple).

Self-provided test equipment required for test (not provided, but can assist in purchase):

1. Microplate reader (450nm wavelength filter)
2. The high-precision dispenser and disposable suction of imported brands: 0.5-10ul, 2-20ul, 20-200ul, 200-1000ul

3. 37℃ incubator, double steam water or deionized water, coordinate paper.
Can assist to buy related products, please consult.

Matters need attention:

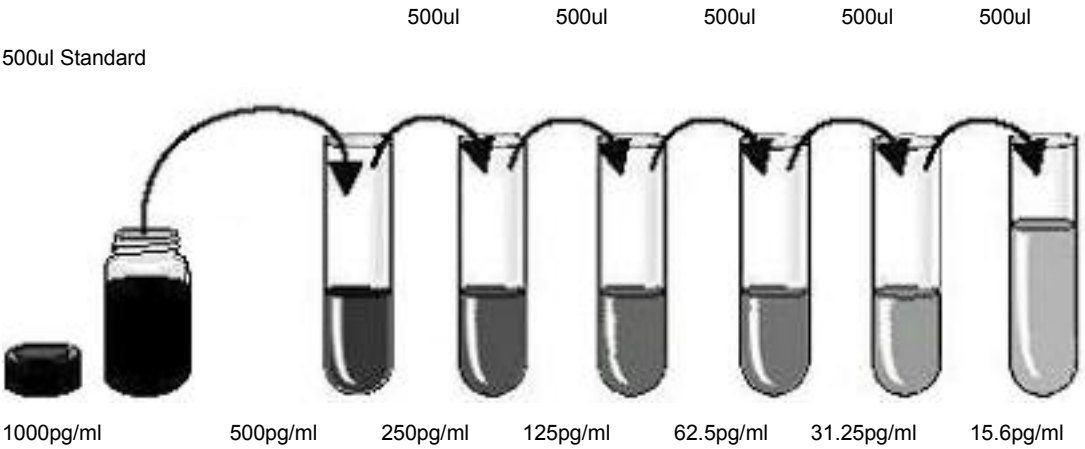
1. Keep the kit at 2-8℃ before use. If the standard is not used up, please it.
2. Concentrate biotinylated antibodies, concentrate enzyme binding volume is small, bumpy and possible inversion in transport, will get the liquid to the tube wall or bottle cap. Therefore, shake the heart several times or 1000 r / p. m. for 1 minute before use to deposit the liquid from the attached tube wall or bottle cap to the bottom of the tube. Before use, carefully blow with the pipette 4-5 times to mix the solution.
3. The concentrated washing liquid taken out from the refrigerator may be crystallized, which is a normal phenomenon. Microheated to 40℃ to dissolve the crystallization completely and then prepare the washing solution.(Heating temperature shall not exceed 50℃) Washing solution shall be room temperature when used.
4. The kit components of different batches should not be mixed (except for washing solution and reaction termination solution). Please consult Xinbosheng Biotechnology Co., Ltd. in advance.
5. Full slight mixing is particularly important for the reaction results, it is best to use a trace oscillator (using the lowest frequency), such as no trace oscillator, can be manually gently hit the microplate frame before the reaction.
6. Double hole test is recommended for standards and samples in the test.
7. The test tube and suction head used in the test are disposable use, **and it is strictly prohibited to mix between different liquids! Otherwise, it will affect the test results.**
8. During the test, please wear test clothes and latex gloves to protect the work. Especially when testing blood or other body fluid specimens, please follow the safety protection list of the National Biological Laboratory.

Preparation work before the test:

1. Please remove the kit from the refrigerator 20 minutes in advance to equilibrate to room temperature.
2. Dilute the concentrated washing solution with double steam water **(1:20)**. Put the unused ones back for 4℃.
3. Standard: Add **1.0ml** of standard & sample to lyophilized standard, let stand for 15 minutes, and gently mix well (concentration **2000 pg / ml**). And then diluted as needed.(It is recommended to use the following concentrations for the standard curve: **1000,500,250,125,62.5,31.25,15.6, and 0 pg/ml**).0 pg / ml is the blank hole. Resolve standard stock solution (**2000 pg/m**l) if not used up.
4. Biotinylated antibody working solution: according to the amount required by the test, concentrate the biotinylated antibody (**1:30**) for 20 minutes into the working concentration. Use on the same day. If the number of experiments leads to the shortage of biotinylated antibodies, biotinylated antibodies can be purchased separately from Xinbosheng Company.(Antibody lot number required)

5. Enzyme binding working solution: according to the amount required by the test, the concentration (1:30) is diluted with the enzyme binding dilution for 20 minutes before use into the working concentration. Use on the same day. If the number of experiments leads to the amount of enzyme concentrate, it can be purchased separately from Xinbosheng Company.(Batch number to be provided)

Standard dilution method legend: (take 500ul / tube, can also be diluted according to the actual amount, such as 200ul / tube)



Washing method:

1. Automatic plate washing machine: the required injected washing liquid is 350ul, with an interval of 30-60 seconds between injection and suction. Wash the plate 5 times.
2. Hand washing board: shake the liquid in the hole, pat dry on the clean absorbent paper, add 350ul of washing liquid to each well, stand for 30 seconds before shake the liquid, and pat dry on the thick absorbent paper. Wash the plate 5 times.

Operating steps:

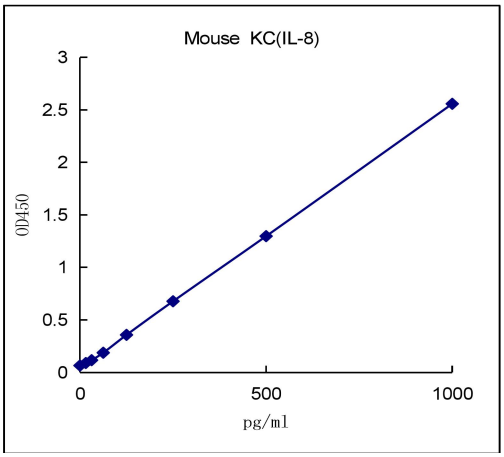
1. Remove the strip required for the test from the sealed bag balanced to room temperature. Put the unused strip and desiccant back into the aluminum foil bag, consolidate the self-sealing strip, seal the pocket, and put it back for 4℃.
2. Add standards and standard sample general dilution, add samples or different concentrations of standards (100ul / well) to the other wells, seal the reaction wells with sealing paper, 37℃ incubator, and incubate from light for 90 minutes.
3. Prepare the biotinylated antibody working solution 20 minutes in advance.
4. Wash the plate for 5 times.
5. Add the biotinylated antibody dilution solution to the blank wells, and the biotinylated antibody working solution (100ul / well) is added to the other wells. The reaction wells were sealed with freshly sealed sheet tape and incubated in a 37℃ incubator for 60 min protected from light.
6. Prepare the enzyme binding solution 20 minutes in advance. Place away at room temperature (22-25℃).

7. Wash the plate for 5 times.
8. Add the enzyme conjugate dilution to the blank wells, and add the enzyme conjugate working solution (100ul / well) to the remaining wells. The reaction wells were sealed with freshly sealed sheet tape, and incubated for 30 min protected from light.
9. Turn on the power supply of the microplate meter, preheat the instrument, and set up the detection procedure.
10. Wash the plate for 5 times.
11 Add chromogenic substrate (TMB) 100ul / well, 37℃ incubator and incubate for 15 min.
12. Add 100ul / well and measure OD 450 (within 3 minutes) immediately after mixing. Save the reading results in the instrument and print a copy of the paper results.
13. After the experiment is completed, put the unused reagent back to the refrigerator according to the specified storage temperature until the end of the expiration date.

It is recommended to preserve the microplate frame for the next time or for future testing.

Results judgment:

1. The OD of each standard and specimen shall subtract the OD of the blank hole.
2. The concentration of the standard is used as the abscissa, and the OD value is used as the ordinate. The best fitting curve is manually drawn or drawn by software to select the best fitting curve. It is recommended to use the quadratic polynomial equation for fitting. The concentration of the OD value of the specimen can be found on the standard curve.
3. If the OD value of the specimen is above the upper limit of the standard curve, it should be appropriately diluted and retest, and the concentration should be multiplied by the dilution multiple.



Note: This drawing is for reference only, and the specimen content shall be calculated from the standard curve of the same test standard.

Sensitivity, specificity, and repeatability:

- Sensitivity: minimum measurable mouse KC (IL-8) up to 7.8 pg/ml.
- Specificity: It does not react with mIL-1,2,3,4,5,6,10, IFN, TNF and hIL-8, rIL-8.
- Repeatability: within the plate, the coefficient of variation between the plates is <10%.