Product Information Sheet

Rat GM-CSF ELISA Kit

Catalog No. EK0366

Size 96T

Range 15.6pg/ml-1000pg/ml

Sensitivity < 1pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Storage

Store at 4℃ for frequent use, at -20℃ for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration

Four months at 4℃ and eight months at -20℃.

Application

For quantitative detection of rat GM-CSF in sera, plasma, body fluids, tissue lysates or cell culture supernates.

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Principle

Antagene's rat GM-CSF ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat GM-CSF specific-specific polyclonal antibodies were precoated onto 96-well plates. The rat specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the rat GM-CSF amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant rat GM-CSF standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- rat GM-CSF antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- rat GM-CSF antibody : 130μl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130μl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

   Preparation of 0.01M **TBS:** Add 1.2g Tris, 8.5g Nacl; 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

   Preparation of 0.01 M **PBS:** Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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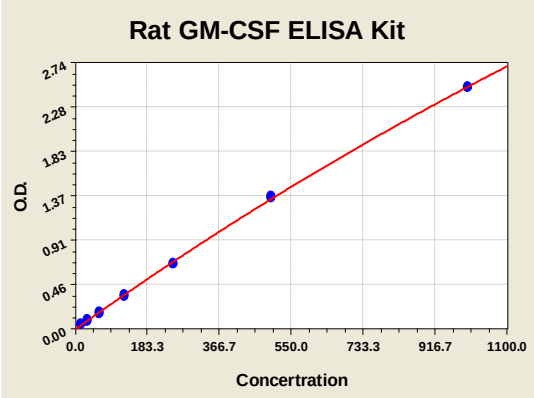
Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃ for 30 min before using.

Rat GM-CSF ELISA Kit-1X96 Well Plate Image



Background

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is also symbolized CSF2. Human GM-CSF is a glycoprotein that is essential for the in vitro proliferation and differentiation of precursor cells into mature granulocytes and macrophages. The human cDNA clones contain a single open-reading frame encoding a protein of 144 amino acids with a predicted molecular mass of 16,293 daltons and show 69% nucleotide homology and 54% amino acid homology to rat GM-CSF. The gene for human GM-CSF appears to exist as a single-copy gene.[1] Human GM-CSF is a 22,000-dalton glycoprotein that stimulates the growth of myeloid progenitor cells and acts directly on mature neutrophils. The GM-CSF gene is localized by somatic cell hybrid analysis and in situ hybridization to human chromosome region 5q21-5q32, which is involved in interstitial deletions in the 5q- syndrome and acute myelogenous leukemia.[2] A complementary DNA for the T lymphocyte-derived lymphokine, GM-CSF has been cloned, and recombinant GM-CSF protein has been expressed in yeast and purified to homogeneity. This purified human recombinant GM-CSF stimulates peripheral blood monocytes in vitro to become cytotoxic for the malignant melanoma cell line A375.[3] The standard product used in this kit is recombinant rat GM-CSF, consisting of 125 amino acids with the molecular mass of 14.8KDa.

Reference

1. Cantrell, M. A.; Anderson, D.; Cerretti, D. P.; Price, V.; McKereghan, K.; Tushinski, R. J.; Mochizuki, D. Y.; Larsen, A.; Grabstein, K.; Gillis, S.; Cosman, D. Cloning, sequence, and expression of a human granulocyte/macrophage colony-stimulating factor. *Proc. Nat. Acad. Sci.* 82: 6250-6254, 1985.
2. Huebner, K.; Isobe, M.; Croce, C. M.; Golde, D. W.; Kaufman, S. E.; Gasson, J. C. The human gene encoding GM-CSF is at 5q21-q32, the chromosome region deleted in the 5q- anomaly. *Science* 230: 1282-1285, 1985.
3. Grabstein, K. H.; Urdal, D. L.; Tushinski, R. J.; Mochizuki, D. Y.; Price, V. L.; Cantrell, M. A.; Gillis, S.; Conlon, P. J. Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. *Science* 232: 506-508, 1986.