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CL199P Polyclonal Antibody to Asialoganglioside GM1 - Ig Fraction

Alternate names: Asialo GM1, GA1

Quantity: 1 ml

Concentration: Antibody Titer: Approximately 1/1000 by Immunoflocculation test. Total Protein Concentration: 37 mg/ml. Albumin Concentration: 9 mg/ml.

Background: Gangliosides are neuraminic acid containing glycosphingolipids and represent characteristic constituents of the plasma membrane of eukaryotic cells. They are shed in the microenvironment and found as free components in plasma. In turn, free gangliosides are efficiently incorporated into the plasma cell membrane.

Host / Isotype: Rabbit / IgG

Immunogen: Asialo GM1 purified from Bovine brain tissue, methylated BSA and complete Freund's adjuvant.

Format: **State:** Lyophilized Ig fraction of serum

Purification: IgG fraction of Serum was obtained by 50% Ammonium Sulfate Precipitation followed by Dialysis with PBS, pH 7.2
Reconstitution: Restore with 1 ml distilled water. Since the material is lyophilized with salts, use of other solvents such as PBS or MEM may increase the salt concentration.

Applications:
Flow Cytometry.
Immunohistochemistry on Frozen Sections.
Immunohistochemistry on Paraffin Embedded Sections.
Immunoprecipitation.
NK Cell Depletion.

Injections:
Mouse - intravenously: 10-50 µl (approximately 20 µl; 50 injections can be made using 20 µl doses). The exact dosage should be decided from titration data (See "**Protocols**") and the nature of the study. The first injection may be effective for 4 days with a gradual diminution. Therefore, 3-4 injections are necessary for a 2 week study. (Incubation) Days / Injection: 0 / 1st, 5 / 2nd, 10 / 3rd, 14 / 4th.
* 50 injections can be made using 20 µl doses.
Rat - intravenously: 50-250 µl (4 or 5 times the usual mouse dose is required). Health conditions and weight of rats should be taken into consideration. It is recommended that the researcher assay NK activity to determine the proper dosage.
Mouse and rat - intraperitoneally: Dosage should be equal to or greater than the i.v. dosage.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

Anti-Asialo GM1 polyclonal antibody reacts with Natural Killer (NK) cells.
It also exhibits slight reactivity with mouse monocytes (liver cells which contain no NK cells; bone marrow; fetal liver cells; spleen cells of nude mice), macrophages, and fetal thymocytes (12 days old; ratio of existence decreased gradually until there were none in newborn mice).
Anti-Asialo GM1 antiserum has been shown to eliminate NK activity in cells of various strains of mice and rats.
Species: Mouse and Rat.
Other species not tested.

Add. Information:

Ig Classes of the antibody: IgG, IgA, and IgM.
Note: Since this antibody is not an antigen affinity purified product, the protein concentration is not equal to the concentration of Asialo-GM1-specific antibody, even after correcting for the Albumin content. Both the total protein and Albumin concentrations will vary from lot to lot.

Storage:

Store the antibody at 2-8°C before reconstitution.
After reconstitution store at 2-8° for up to 2-3 months.
DO NOT FREEZE.
Recontstituted product is stable for 2 days at RT.

Product Citations:

Purchased from Acris:

1. Kinpara S, Hasegawa A, Utsunomiya A, Nishitsuji H, Furukawa H, Masuda T, et al. Stromal cell-mediated suppression of human T-cell leukemia virus type 1 expression in vitro and in vivo by type I interferon. J Virol. 2009 May;83(10):5101-8. doi: 10.1128/JVI.02564-08. Epub 2009 Mar 4. PubMed PMID: 19264779.
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General Readings:

1. Godeny EK, Gauntt CJ. Murine natural killer cells limit coxsackievirus B3 replication. J Immunol. 1987 Aug 1;139(3):913-8. PubMed PMID: 3036947.
2. Habu S, Fukui H, Shimamura K, Kasai M, Nagai Y, Okumura K, et al. In vivo effects of anti-asialo GM1. I. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. J Immunol. 1981 Jul;127(1):34-8. PubMed PMID: 7240748.
3. Kasai M, Iwamori M, Nagai Y, Okumura K, Tada T. A glycolipid on the surface of mouse natural killer cells. Eur J Immunol. 1980 Mar;10(3):175-80. PubMed PMID: 6966574.
4. Okamura, K. and Y. Ochali: Metabolism. 17, 47 (1980). (In Japanese).
5. Salazar-Matcher, Orange and Biron: J. Exp. Med. 187 (1998) 1-14.
6. Nishiyama, Fuchimoto and Orita: Jpn. J. Cancer Res. 80 (1989) 366-372.
7. Basse, P. Hokland, Gundersen and M. Hokland: APMIS. 100 (1992) 202-208.
8. Wiltrout, Santoni, Peterson, Knott, Overton, Herberman and Holden: Journal of Leukocyte Biology. 37 (1958) 597-614.
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10. Keilbaugh SA, Shin ME, Banchereau RF, McVay LD, Boyko N, Artis D, et al. Activation of RegIIIbeta/gamma and interferon gamma expression in the intestinal tract of SCID mice: an innate response to bacterial colonisation of the gut. Gut. 2005 May;54(5):623-9. PubMed PMID: 15831905.
11. Rosato A, Zoso A, Milan G, Macino B, Dalla Santa S, Tosello V, et al. Individual analysis of mice vaccinated against a weakly immunogenic self tumor-specific antigen reveals a correlation between CD8 T cell response and antitumor efficacy. J Immunol. 2003 Nov 15;171(10):5172-9. PubMed PMID: 14607917.

Protocols:

<u>Procedure For Measurement of Anti-NK Cell Activity</u>

IN VITRO:

1. Preparation of target cells:
- Suspend 5 x 10^6 cells of YAC-1 in RPMI 1640 containing 10% FCS.
2. Preparation of effector cells:
- Inject 0.2 ml of polyinosinic- polycytidylic acid soduim salt solution (500 µg per ml of poly I:C in RPMI 1640) into BALB/c mice.
- Remove spleens from mice on the following day (after approximately 10 hours of treatment) and prepare spleen cell suspension as follows:
- Centrifuge at 1000 rpm for 10 minutes and discard supernatant.
- Add 0.83% NH_4Cl to the tube to hemolyze the precipitated spleen cells.
- Centrifuge at 1000 rpm for 10 minutes and discard supernatant. Add 10 ml RPMI 1640 to wash cells.
Repeat washing process using same procedure.
- Adjust the cell number to 2.5 x 10^7 cells per ml.
3. Treatment of effector cells:
- Dilute the target cell suspension with RPMI 1640 to ratios of 1:50, 1:100, and 1:200.
- Place 0.5 ml of effector cell suspension into centrifugation tubes. Centrifuge at 1000 rpm for 10 minutes and discard supernatant.
- Add 0.5 ml of the diluted target cell suspension to each tube above and mix well.
- Incubate tubes at 37°C for 30 minutes in 5% CO_2.
- Centrifuge at 1000 rpm for 10 minutes and discard supernatant.
- Prepare control by adding 0.5 ml of RPMI 1640 to effector cells. Mix well to make suspension.
- Make a dilution of Guinea Pig Complement with RPMI 1640 and add the diluted complement to effector cell suspensions. Mix well.
- Incubate at 37°C for 30 minutes with occasional stirring.
- Centrifuge at 1000 rpm for 10 minutes and discard supernatant.
- Add 1 ml of inactivated FCS (10% FCS in RPMI 1640) and mix well.
4. Measurement of activity (n=2):
- Place 100 µl of the target cell suspension into each well of microplate.
- Place 100 µl each of the untreated samples, the diluted samples, and the samples of effector cells with complement into each well.
For measurement of spontaneous Cr release, add 100 µl of RPMI 1640 containing inactivated 10% FCS to well.
- For measurement of maximum Cr release, centrifuge 0.5 ml of target cell suspension at 1000 rpm for 5 minutes. Discard supernatant and add 1.0 ml of sterile water.
- Cover the microplate and tubes. Incubate at 37°C for 10 hours in 5% CO_2.

- Assay the radioactivity of 100 µl of each reaction mixture using an autogamma counter.
- Calculate the % Lysis using the following equation:
% Lysis =
Experimental Cr release - Spontaneous Cr release x 100
Maximum Cr release- Spontaneous Cr release

IN VIVO:
1. Preparation of target cells:
- Suspend 5 x 10^6 cells of YAC-1 in RPMI 1640 containing 10% FCS.
2. Preparation of effector cells:
Dilute the sample with RPMI 1640 to ratios of 1:2, 1:4, and 1:8
- Inject BALB/c mice with diluted samples in 0.2 ml doses (n=3).
- Dilute rabbit serum to 1:2 with RPMI 1640 and inject into BALB/c mice in 0.2 ml doses.
- After 3 days of treatment, remove spleens and prepare suspensions using RPMI 1640 as follows:
- Centrifuge at 1000 rpm for 10 minutes and discard supernatant.
- Add 0.83% NH_4Cl to the tubes to hemolyze the precipitated spleen cells.
- Centrifuge at 1000 rpm for 10 minutes and discard supernatant.
- Add 10 ml of RPMI 1640 to the tubes to wash the precipitated cells. Repeat washing process using same procedure.
- Centrifuge at 1000 rpm for 10 minutes and collect the cells. Add RPMI 1640 containing inactivated 10% FCS to the tubes to adjust the cell number to 2.5 x 10e7 cells per ml.
3. Measurement of activity:
- Follow same procedure as outlined in Section #4 under ***IN VITRO***.

Indirect Immunoperoxidase Method
FROZEN SECTION (6 µm)
1. Washed with PBS buffer (pH 7.2) for 10 min. (X3).
Added to PBS containing 0.05% gluteraldehyde.
2. Fixed for 5 min. at 4°C.
3. Washed with PBS buffer for 5 min.
Added to 100% methanol containing 0.5% H_2O_2 (for removal of endogenous peroxidase activity)
4. Incubated for 30 min.
5. Added to Anti normal goat serum (for avoiding non-specific antigen-antibody reaction).
Added to anti-asialo GM1, Rabbit (CL199P) as first antibody.
6. Incubate for one hour at room temperature, or overnight at 4°C.
7. Washed with PBS buffer for 5 min. (X3).
Added to anti-rabbit peroxidase goat IgG as second antibody.
8. Incubated for 30 min. - 3 h.
9. Washed with PBS buffer (X3).
Added to 100 ml of Karnowsky substrate solution (Karnowsky substrate solution:

0.05M Tris-HCl buffer, pH 7.6 100 ml / Diaminobenzidine tetrachloride 0.25 mg / 5% Hydrogen peroxide 0.1 ml)
10. Incubated at RT for 3-30 min.
11. Transferred to distilled water (to stop)
Added to 1% OsO_4 (Osmic Acid Solution 1%) for 2-3 sec.
Stained with methyl green.
Dehydrated with dilution series of ethanol.
Treated with xylene.
Embedded with balsam.
Control:
1. First antibodies were omitted.
2. First antibodies were replaced with non-immune sera of the same species as the specific antiserum.
3. DAB reaction only.
mounted with Hematoxylin-Eosin staining

Titration of Anti-Asialo GM1 *in ViVo*
Amount of Anti-Asialo GM1 i.v. injected into BALB/c mice vs. **% Lysis against YAC-1 cells by spleen cells taken 3 days after a 1-shot injection (Effector/target 50:1)**:
10 µl / 2.1 % Lysis
25 µl / 1.8 % Lysis
50 µl / 1.1 % Lysis
100 µl / 1.0 % Lysis
Normal rabbit serum injected: 100 µl / 20.6 % Lysis against YAC-1 cells
NOTE: BALB/c mice were injected with 100 µg of polyinosinic-polycytidylic acid sodium salt (0.2 ml of 500 µg/ml Poly I:C) and maintained for 18 hours before next procedure.

Pictures:

Titration of Anti-Asialo GM1 in vitro:
Spleen cells of BALB/c were treated with Anti-Asialo GM1 and Guinea Pig Complement. Remaining NK activities were tested in vitro by using YAC-1 cells as target. Effector/target ratio was 50:1. O represents NK activities of BALB/c spleen cells treated with complement.