GENTAUR

DATA SHEET

ANTI ASIALO GM1 (RABBIT)

Cat. No. 986-10001

Anti Asialo GM1 antiserum has been shown to eliminate natural killer (NK) activity in cells of various strains of mice and rats.

Preparation: Purified asialo GM1 from bovine brain tissue was repeatedly immunized with methylated bovine serum albumin and with complete Freund's adjuvant. Gammaglobulin fraction of serum was obtained by 50% ammonium sulfate precipitation methods followed by dialyzing with phosphate buffered saline (pH 7.2).

Specificity: Reacts with mouse and rat NK cells; mouse monocytes (liver cells which contain no NK cells; bone marrow; fetal liver cells; spleen cells of nude mice macrophages); fetal thymocytes (12 days old; ratio of existence decreased gradually until there were none in newborn mice).

Immunoglobulin fractions: IgG, IgA, and IgM

Contents: 1 mL

Storage: 2-10°C

Stability: Before reconstitution: 2 years when refrigerated.
After reconstitution: 2-3 months when refrigerated, 2-3 days at room temp.
DO NOT FREEZE

Reconstitution: Distilled water is recommended (1 mL). Since the material is lyophilized with salts, use of other solvents such as PBS or MEM may increase the salt concentration.

Antibody titer: Approximately 1:1000 by immunoflocculation test.

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Injections:

Mouse - intravenously: 10-50 μL (approximately 20 μL). The exact dosage should be decided from titration data enclosed with package (please see following page) 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	0	5	10	14
	^	^	^	^
Injection	1st	2nd	3rd	4th

* 50 injections can be made using 20 μL doses.

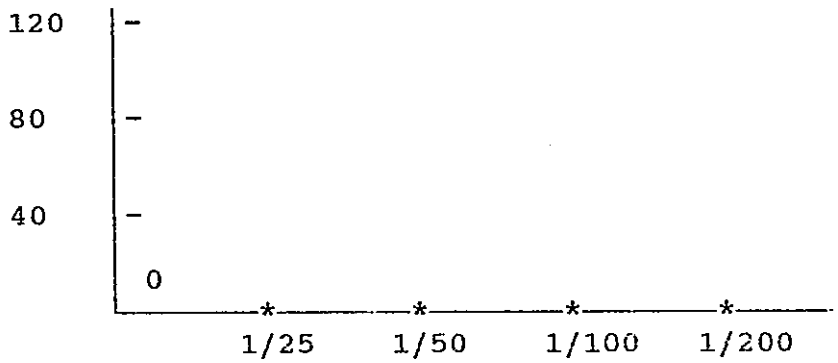
Rat - intravenously: 50-250 μL (4 or 5 times the usual mouse dose is required. Health conditions and weight of rats should be taken into consideration. It is recommended that the researcher assay NK activity to determine the proper dosage to inject.

Mouse and rat - intraperitoneally: Dosage should be equal to or greater than the i.v. dosage.

Each package comes with *in vivo* and *in vitro* titration data for the lot. For example:

ANTI ASIALO GM1 (RABBIT)
Product Code No. 986-10001
Lot No. PDK9700

Titration of Anti Asialo GM1 *in vitro*



Spleen cells of BALB/c* were treated with Anti Asialo GM1 and guinea pig complement. Remaining NK activity was tested *in vitro* using YAC-1 c-lls as target (represented by *----* in above graph). Effector/target ratio was 50:1. 0 represents NK activities of nu/nu spleen cells treated with complement.

Titration of Anti Asialo GM1 *in vivo*

Amount of Anti Asialo GM1 injected into BALB/c* mice	% Lysis against YAC-1 cells by spleen cells taken 3 days after a 1-shot injection (Effector/target 50:1)
10 μL	7.0
25 μL	8.4
50 μL	2.0
100 μL	0.0
Normal rabbit serum injected	% Lysis against YAC-1 cells
100 μL	70.8

* Note: BALB/c mice were injected with 100 μg of polyinosinic-polycytidylic acid soduim salt (0.2 mL of 500 μg/mL Poly I:C) and maintained for 18 hours before next procedure.

Procedure For Measurement of Anti NK Cell Activity

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 sodium 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 sample 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 samples 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.

   - Dilute guinea pig complement 10 times 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:

$$\% \text{ Lysis} = \frac{\text{Experimental Cr release - Spontaneous Cr release}}{\text{Maximum Cr release - Spontaneous Cr release}} \times 100$$

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 sample and diluted sample 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 as above.
   - 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 10^7 cells per mL.

3. **Measurement of activity:**
   - Follow same procedure as outlined in section #4 under *"IN VITRO."*

REFERENCES:

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