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AM26416PU-N Monoclonal Antibody to KOR-SA3544 - Purified

Quantity: 0.1 mg

Background: The Philadelphia chromosome (Ph1) has been implicated as the causative factor in greater than 90% of chronic myelogenous leukemia (CML), in 25–30% of adult and 2–10% of childhood acute lymphoblastic leukemia (ALL) and in rare cases of acute myelogenous leukemia (AML). The presence of the Ph in leukemic cells of ALL patients usually indicates poor prognosis and high risk. Sequential monitoring of the Ph in ALL correlates with the activity of malignant clones and predicts impending clinical relapse, and therefore is useful in guiding clinical therapeutic decisions.

Host / Isotype: Mouse / IgG1

Recommended Isotype Controls: SM10P (for use in human samples), AM03095PU-N

Clone: KOR-SA3544

Immunogen: Cell line (KOCL-22) established from bone marrow blood of patient with congenital leukemia

Format:
State: Lyophilized Ig fraction
Purification: Protein A agarose
Buffer System: PBS (pH 7.2) containing 1% sucrose
Preservatives: 0.09% NaN3
Reconstitution: Prepare a stock solution by dissolving the lyophilized antibody in 100 µL of distilled water.

Applications:
Western blot: 10 µg/ml.
Flow cytometry: 10 µg/ml (final concentration).
For deteails see protocols below.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The KOR-SA3544 monoclonal antibody showed reactivity with a surface antigen expressed on the Philadelphia chromosome ((Ph1)-positive acute lymphoblastic leukemia (ALL)) with no exceptions (26/26 cases). The recognized antigen is a nonspecific cross-reacting antigen (NCA)-50/90 (CD66c), one of the carcinoembryonic antigen (CEA)-related glycoproteins encoded by a member of the CEA gene family.

The reactivity of this antibody has been reported as follows:
Common ALL[a] 5/38 (13.2%)
Early B precursor ALL[b] 1[c]/21 (4.8%)
T-ALL 0/19

B-ALL 0/6
B-CLL/HCL 0/3
Multiple myeloma 0/2
ANLL 16/56 (28.6%)

Ph1-ALL 26/26[d] (100%)
CML blastic crisis 0/9
T-NHL 0/5
B-NHL 0/4
Hodgkin's disease 0/1
CLL, chronic lymphocytic leukemia
HCL, hairy cell leukemia
NHL, non-Hodgkin's lymphoma
[a]CD10+, CD19+, HLA-DR+
[b]CD10-, CD19+, HLA-DR+
cOne patient with 11q23 translocation.
[d]Eighteen patients with m-bcr, eight patients with M-bcr

Storage:

Prior to reconstitution store at 2-8°C. Following reconstitution store (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:

1. Sugita, K., et al., Leukemia, 13, 779-785 (1999).
2. Mori, T., et al., Leukemia, 9, 1233-1239 (1995).

Protocols:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate at 4oC while rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with an equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer from membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal the sample in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The

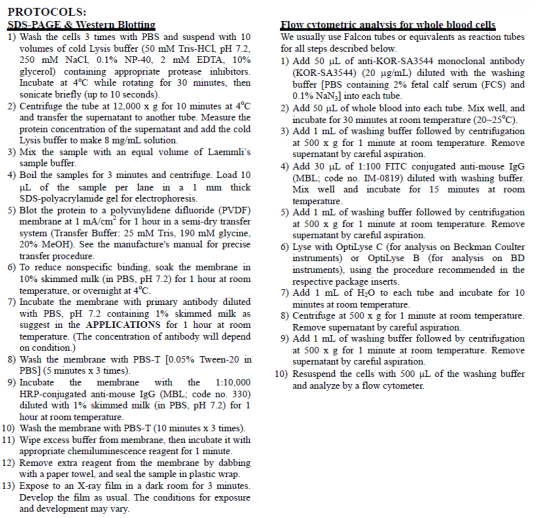
conditions for exposure and development may vary.

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

1) Add 50 µL of anti-KOR-SA3544 monoclonal antibody (KOR-SA3544) (20 µg/mL) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25oC).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
7) Add 1 mL of H2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Pictures:



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9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer from membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
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10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Flow cytometric analysis of KORSA3544 expression on Granulocyte (R1). Open histogram indicates the reaction of isotypic control to the cells. Shadd histogram indicates the reaction of AM26416PU-N to the cells.