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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 [®] 5/38 (13.2%) Early B precursor ALL [®] 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%)

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MS/20140808

	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 40C. 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 PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times). 9) Incubate 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

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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 μ L of the washing buffer and analyze by a flow cytometer.

Pictures:

PROTOCOLS

ROTOCOLS: DSPACE AVErent Blotting Wash the cells 3 times with JPS and suspend with 10 volumes of coll Lysis buffer (50 nM Tin+HCL pH 7.2, 250 mM NaCL 0.1% NP-40, 2 mM EDTA, 10% phycrol) constituing appropriate protess inhibitors by the start of the start of the start of the start sonicate briefly (up to 10 scores). Cleriftigge the the al 12,000 x g for 10 minutes at 34° content for the start and 200 minutes at 34° content occentration of the uperstatut and add the coll Lysis buffer to make 8 mg/mL solutions sample buffer Boil the samples for 3 minutes and centrifuge Load 10 µL of the sample per lane in a 1 mm thick

- mple butter. ol the sample for 3 minutes and centrifuge. Load 10 L of the sample per lane in a 1 mm thick DS-polyacytamized gef for electrophoresis. Iot the protein to a polyaviptidene difficuited (PVDF) embrane at 1 mA/cm for 1 hour in a semi-dyt marker system (Transfer Buffer: 25 mM Tins, 190 mM glycine, 0% MGOH). See the manufacture's manual for precise references buffer.
- rocedure. e nonspecific binding, soak the membrane in mmed milk (in PBS, pH 7.2) for 1 hour at room are, or overnight at 4°C. the membrane with primary antibody diluted the membrane with primary antibody diluted mperature, or overnight at 4°C. cubate the membrane with primary antibody ith PBS, pH 7.2 containing 1% skimmed aggest in the APPLICATIONS for 1 hour
- temperature. (The concentration of antibody will depend 30 Wash the membrane with PBST [0.05% Toren-20 in PBS] (5 minutes x3 times).
 9) Incubate the membrane with the 11.0.000 HRP-conjugated anti-mouse IgG (MBE, code no. 330) dhaled with 1% skimmed mikk in PBS, pH 7.2) for 1
 10) Wash to some integeration. PBS-T (10 minutes x 3 times).
 10) Wash code MBF from membrane the incubate with appropriate chemaluminescence reagent for 1 minute.
 12) Renove extra cogent from the membrane by dabbing with apper towel, and self the sample in plastic wrap. D Expose to an X-ray film as a data from for 3 minutes. In 2 Renove extra X-ray film as a data from for 5 minutes. D Expose to an X-ray film as a data from for 5 minutes. In 3 development may vary.

Env: cytometric nanbrisk for whole blood cells We suadly use schelon mhess or equivalents as reaction nubes 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% feat call sterms (FCS) and 0.1 Add 50 µL of whole blood into each tube. Mix well, and 0.1 Add 50 µL of whole blood into each tube. Mix well, and

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- supernatant by careful aspiration. Add 30 µL of 1:100 FITC conjug (MBL; code no. IM-0819) diluted Mix well and incubate for 15
- temperature. 5) Add 1 nut. of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatura by careful aspiration. 6) Lyse with OptLyse C for analysis on Beckman Coulter instruments), using the procedure recommended in the respective package inserts. 7) Add 1 nut. of Fig.0 to each tube and incubate for 10 minutes at room temperature.
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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.



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