For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-CD66c (KOR-SA3544) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D028-3	KOR-SA3544	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: This monoclonal antibody (clone KOR-SA3544) was reactive with a surface antigen expressed on Philadelphia chromosome (Ph1)-positive acute lymphoblastic leukemia (ALL) without exception (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 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.

SOURCE: This antibody was purified from hybridoma (clone KOR-SA3544) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell 63.Ag8.653/NS1 with Balb/c mouse splenocyte immunized with a cell line (KOCL-22) established from bone marrow blood of patient with congenital leukemia.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: 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]$CD10^+$, $CD19^+$, $HLA\text{-}DR^+$
[b]$CD10^-$, $CD19^+$, $HLA\text{-}DR^+$
[c]One patient with 11q23 translocation.
[d]Eighteen patients with m-bcr, eight patients with M-bcr

APPLICATIONS:
Western blotting; 10 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

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

Clone KOR-SA3544 is used in these references.

RELATED PRODUCTS:
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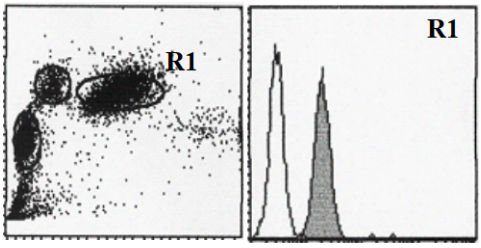
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C 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 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/cm^2 for 1 hour in a semi-dry transfer

system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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 4°C.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested 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).
9) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (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).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.



Flow cytometric analysis of KOR-SA3544 expression on Granulocyte (R1). *Open histogram indicates the reaction of isotypic control to the cells. Shadd histogram indicates the reaction of D028-3 to the cells.*

Flow cytometric analysis for whole blood cells

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

1) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer (PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3) into each tube.
   *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
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 FITC-conjugated anti-mouse IgG antibody-FITC 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, Beckman Coulter, code no. A11895) or OptiLyse B (for analysis on BD instruments, Beckman Coulter, code no. IM1400), using the procedure recommended in the respective package inserts.
7) Add 1 mL of H_2O 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.