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Product datasheet for AM26473AF-N

CXCR4 (extracell. Dom.) (N-term) Rat Monoclonal Antibody [Clone ID: A145]

Product data:

Product Type:	Primary Antibodies
Clone Name:	A145
Applications:	FC
Recommended Dilution:	Flow cytometry: 10-20 µg/ml (final concentration). For details see protocols below.
Reactivity:	Human
Host:	Rat
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Cos-1 cells transfected with CXCR4 gene
Specificity:	The antibody recognizes the N-terminal, extracellular domain of the CXCR4 protein.
Formulation:	PBS containing 50% glycerol. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Ammonium sulfate precipitation followed by gel filtration through Superdex 200 in PBS
Conjugation:	Unconjugated
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	C-X-C motif chemokine receptor 4
Database Link:	Entrez Gene 7852 Human P61073
Synonyms:	CXC-R4, CXCR-4, Fusin, LCR1, FB22, NPYRL, HM89, SDF1 receptor, LESTR
Note:	This product was originally produced by MBL International.

Protocol:
Flow cytometric analysis for floating cells

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Protocol 1

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 to the cell pellet after tapping. Mix well, and incubate for 5 minutes at room temperature (20~25 oC).
5) Add 30 µL of the anti-CXCR4 monoclonal antibody (10-20 µg/mL) diluted with the washing buffer. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
6) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
7) Add 30 µL of secondary antibody (1:40 FITC conjugated anti-Rat IgG) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature (20~25oC).
8) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Protocol 2
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with PBS containing 25% normal goat serum, 1 mg/mL normal human IgG and 0.1% NaN3 (5x106 cells/mL).
3) Add 20 µL of the anti-CXCR4 monoclonal antibody (50 µg/mL) diluted with the washing buffer into each tube.
4) Add 50 µL of the cell suspension into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
5) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
6) Resuspend the cells with 50 µL of the washing buffer.
7) Add 20 µL of secondary antibody (1:10 FITC conjugated anti-Rat IgG) diluted with the washing buffer into each tube. Mix well and incubate for 15 minutes at room temperature (20~25oC).
8) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.