

Product datasheet for **AM39033FC-N**

Human Kappa Light Chain (free and bound) Mouse Monoclonal Antibody [Clone ID: NaM76-5F3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	NaM76-5F3
Applications:	FC
Recommended Dilution:	Flow cytometry - for analysis of blood samples. Rabbit anti-human kappa light chain has been selected for use in combination with anti-human lambda, with CD19, CD138 or as a triple reagent i.e. anti-kappa plus anti-lambda plus CD19 or CD138. Please see "Protocols" below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Specificity:	The antibody reacts with free light chains as well as intact immunoglobulin molecules. This reagent is especially valuable in the study of the monoclonal nature (light chain restriction) of lymphoid neoplasms.
Formulation:	0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, 0.2% BSA, 0.09% sodium azide Label: FITC State: Liquid purified Ig fraction Label: <u>Cat. No. Label EX-max (nm) / EM-max (nm)</u> : AM39033FC-N FITC 488 / 519 AM39033RP-N 488, 532 / 578
Concentration:	lot specific
Purification:	Affinity chromatography
Conjugation:	FITC
Storage:	Store the antibody undiluted at 2-8°C. Fluorochrome labelled product is photosensitive and should be protected from light.
Stability:	Shelf life: one year from despatch.



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Background:

Kappa light chains of human immunoglobulins occur in 50-70% of normal human B lymphocytes while lambda light chains are expressed in 30-50% of these cells. Abnormal expression of kappa and lambda light chains occur in leukemia. Anti-kappa and anti-lambda antibodies are frequently used in combination with CD19 or CD138 for the detection of light chains of surface immunoglobulin on normal and neoplastic B cells.

Note:

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.
3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Protocol: Flow cytometry method for use with labelled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) monoclonal antibodies

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of labelled monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study). Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Add 100 µl of a lyse reagent.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 µl of PBS.
9. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Flow cytometry method for use with dual and triple combinations

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.

For combinations with anti-kappa and/or anti-lambda Ig see **Application note** below.

2. Add to each tube 20 µl of labelled monoclonal antibody combination. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of a lyse reagent and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.

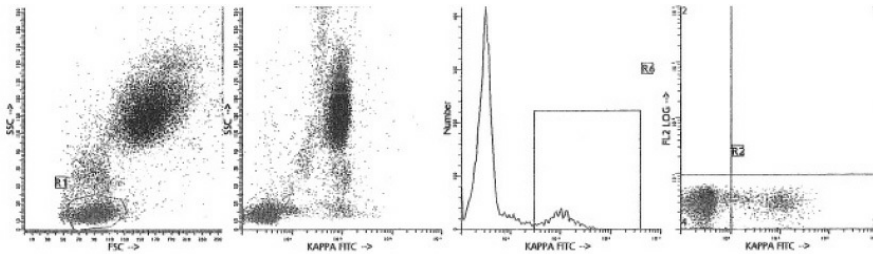
8. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.
10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Application note for anti-kappa and/or anti-lambda Ig combinations

Add 2 ml of PBS containing 0.001% (v/v) Heparin (prewarmed to 37°C) to the cell suspension Vortex, centrifuge (2 min at 300x g) and discard the supernatant.

Repeat this step twice.

Resuspend the pelleted blood cells in 100 µl PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Product images:

Representative Data The reactivity of anti-kappa is illustrated by flow cytometry analysis of normal human peripheral blood cells. The cytograms show direct staining of 100 I whole blood with 10 I of anti-kappa FITC.