

PRODUCT INFORMATION SHEET
 Monoclonal antibodies detecting human antigens

Anti-BrdU

PURE	<input type="checkbox"/> RUO	<input type="checkbox"/> REF	IQP-170P	▽	100 tests
FITC	<input type="checkbox"/> RUO	<input type="checkbox"/> REF	IQP-170F	▽	100 tests

RUO *For Research Use Only*



Description

Clone Mobu-1

Isotype Murine IgG1

Specificity Clone Mobu-1, anti-BrdU is an IgG1 monoclonal antibody which specifically binds to 5-bromo-2'-deoxy-uridine and shows no cross reactivity with 5'-fluoro-2-deoxy-uridine, or any endogenous cellular components, such as thymidine or uridine.

Antigen distribution

The measurement of DNA synthesis or cell proliferation is important in cell biology research. Anti-BrdU is used to detect BrdU that has been incorporated into the DNA during the S-phase of proliferating cells [1].

Applications

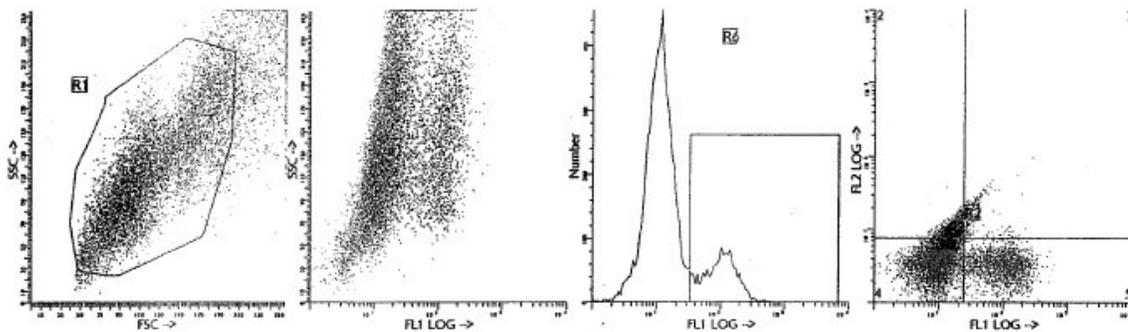
Detection of BrdU incorporation is used to quantify proliferating cells. BrdU is administered in vivo or added to the cell culture for a pre-determined period of time and subsequently detected with anti-BrdU monoclonal antibody. The bound antibody is detected using an enzyme or fluorochrome-conjugated second antibody.

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µL/10⁶ leukocytes for singles and 20 µL/10⁶ leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative Data

Staining with clone Mobu-1 (anti-BrdU) monoclonal antibodies is illustrated by flow cytometry analysis of BrdU incorporated U266. Direct staining was performed using 10 µl of the FITC-conjugated antibody and 150 µl cell suspension (special protocol).



Limitations

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. 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. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
3. Reagents can be used in different combinations, therefore laboratories need to become familiar performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples.
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Reagents and materials required but not supplied

1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. BrdU labeling reagent. Sterile. Dilute to final concentration 10 μ M BrdU in the cell culture medium. Incubation time will depend on the experimental design
7. Washing buffer (PBS + 0,5 % FCS)
8. Anti-BrdU
9. Anti-mouse Ig FITC

Procedure

1. Adjust the cell suspension to 5×10^6 cells per ml with 70% cold ethanol.
2. Incubate for 20 minutes at room temperature.
3. Transfer 150 μ l cell suspension to 3 ml tubes for flow cytometry.
4. Add 2 ml wash buffer and centrifuge for 5 minutes at 400 x g.
5. Remove the supernatant and resuspend the cells in 150 μ l 2M HCl (prepare freshly).
6. Incubate for 20 minutes at room temperature.
7. Add 2 ml wash buffer and centrifuge for 5 minutes at 400 x g.
8. Remove the supernatant and resuspend the cells in 1,5 ml sodium borate (0,1 M pH 8,5).
9. Incubate for 2 minutes at room temperature.
10. Centrifuge for 5 minutes at 400 x g.
11. Remove the supernatant and resuspend the cells in 2,0 ml wash buffer.
12. Centrifuge for 5 minutes at 400 x g.
13. Remove the supernatant.
14. Add 10 μ l of purified or FITC conjugated anti-BrdU monoclonal antibody. Incubate for 20 minutes at room temperature in the dark.
15. Add wash buffer and centrifuge for 5 minutes at 400 x g.
16. Remove the supernatant.
17. If an unconjugated anti-BrdU was used, add 50 μ l rabbit anti-mouse FITC (IQP-190F).
18. Add wash buffer and centrifuge for 5 minutes at 400 x g.
19. Remove the supernatant and resuspend the cells in 300 μ l of PBS.
20. Analyze by flow cytometry .



Handling and Storage

Antibodies are supplied either as 100 tests per vial (1 ml) for singles or 50 tests per vial (1 ml) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN₃). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.

References

1. Gratzner et al., 1983. Proc. Natl. Acad. Sci. 80., 5573
2. Endl, E., et al 1997. Cytometry, 29., 233-241
3. Vanderlaan, M., & Thomas. C.B., 1985. Cytometry. 6. 501-505
4. Ellwart, J., Dormer, P., 1985 Cytometry. 6., 513-520

Explanation of used symbols

	Consult instructions for use
	Catalogue number
	Sufficient for
	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer
	Authorized Representative in the European Community
	Conformité Européenne (European Conformity)

IQ Products
bright fluorescence

		Label - tandem	Ex -max (nm)	Em -max (nm)
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
C	CyQ	PE-Cy5.18	488, 532	667
A	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695



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