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Product datasheet for AM01025FC-N

Bromodeoxyuridine / BrDU Mouse Monoclonal Antibody [Clone ID: BU20a]

Product data:

Product Type:	Primary Antibodies
Clone Name:	BU20a
Applications:	FC
Recommended Dilution:	**Flow Cytometry:** Use Neat-1/10 diluted antibody to label 1x10^6 cells in 100 µl. ***See Recommended Protocol below.***
Reactivity:	Broad
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Bromodeoxyuridine conjugated to BSA.
Specificity:	This antibody recognises the thymidine analogue Bromodoxyuridine (BrdU), which can be incorporated into DNA during S-phase of the cell cycle. The BU20a antibody is suitable for detecting incorporated BrdU in a wide variety of cell types and is suitable for use on tissue sections in double-labelling techniques.
Formulation:	PBS, pH 7.4 Label: FITC State: Liquid purified IgG fraction from Tissue Culture Supernatant Preservative: 0.09% Sodium Azide
Concentration:	lot specific
Purification:	Affinity Chromatography on Protein G
Conjugation:	FITC
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.

Background:

The immunocytochemical detection of bromodeoxyuridine (BrdU) incorporated into DNA is a powerful tool to study the cytokinetics of normal and neoplastic cells. In vitro or in vivo labeling of tumor cells with the thymidine analogue BrdU and the subsequent detection of incorporated BrdU with specific anti-BrdU monoclonal antibodies is an accurate and comprehensive method to quantitate the degree of DNA-synthesis.
BrdU is incorporated into the newly synthezised DNA of S-phase cells may provide an estimate for the fraction of cells in S-phase. Also dynamic proliferative information such as the S-phase transit rate and the potential doubling time can be obtained, by means of bivariate BrdU/DNA flow cytometric analysis.

Note:

Protocol: **Flow Cytometry Analysis**
Prepare the following solutions before proceeding:
Phosphate buffered saline (PBS)
2N HCl, 0.5% Triton X-100
PBS containing 0.05%Tween-20
PBS containing 1%BSA (PBS/BSA)
10mg/ml Propidium iodide (PI)

1: Add BrdU to the cell suspension in culture medium to a final concentration of 10 µmol/L and incubate for 30 minutes in a CO2 incubator at 37°C.
2: Wash cells twice with PBS/BSA, and resuspend in PBS
3: Add cells slowly into 5ml of 70% ethanol at -20°C, mixing continuously (vortex preferred). Incubate on ice for 30 minutes.
4: Centrifuge at 500g for 10 minutes, decant supernatant, and resuspend cell pellet.
5: Add 2ml 2N HCl, 0.5% Triton X-100 and incubate the cells for 30 minutes at room temperature (preferably on a rocking platform).
6: Centrifuge at 500g for 10 minutes, decant supernatant and resuspend in 3 mL 0.1M Na2B4O7, pH 8.5
7: Centrifuge at 500g for 10 minutes, decant supernatant and resuspend the cells in PBS/BSA + 0.05% Tween-20. Adjust cell concentration to 1 x 107/ml
8: Aliquot 100ul of cell suspension into required number of 12 x 75mm tubes.
9: Incubate the cells with the monoclonal anti-BrdU at the recommended dilution for 30 minutes at room temperature.
10: Add 2 mls PBS/BSA and centrifuge the cells at 1000rpm for 5 minutes.
11: If a secondary antibody layer is required then decant the wash and incubate the cells with the secondary antibody for 30 minutes at room temperature. If no secondary antibody layer is required then proceed to step 13.
12: Wash the cells after the secondary antibody layer by repeating step 10.
13: Decant off the wash and add 1ml PBS containing 10µg/ml PI (Dilute the 10mg/ml solution of PI 1/1000 in a suitable volume of PBS)
14: Analyse cells by flow cytometry following the manufacturers instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale.