RayBiotech, Inc.

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Certificate of Analysis and Data Sheet

Rat Anti BrdU:FITC

With HRP-conjugated Secondary Antibody

Catalog No.	Target Species	Isotype
DS-MB-00141	Chemical	IgG2a

Synonyms

5- Bromodeoxyuridine

Preparation

Purification: Purified IgG prepared by ion exchange chromatography from tissue culture supernatant

Formulation

Product Type: Monoclonal Antibody
Product Form: Purified IgG conjugated to Fluorescein Isothiocyanate Isomer 1 (FITC) - liquid
Buffer: Phosphate buffered saline
Preservative Stabilizers: 0.01%Sodium Azide, 50%Glycerol

Specificity

DS-MB-00141 recognizes BrdU incorporated into single stranded DNA, attached to a protein carrier and free BrdU. DS-MB-00141 does not cross react with thymidine but does react weakly with chlorodeoxyuridine.

Storage

Store at +4^{o}C or at -20^{o}C if preferred. This product should be stored undiluted.
Storage in frost free freezers is not recommended. This product is photosensitive and should be protected from light. Avoid repeated freezing and thawing as this may denature the antibody. Should this product contain a precipitate we recommend microcentrifugation before use.
Shelf Life: 18 months from date of shipment

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Recommended Protocol

FLOW CYTOMETRY ANALYSIS

Prepare the following solutions before proceeding:
Phosphate buffered saline (PBS)
2N HCl containing 0.5% Triton X-100
PBS containing 0.05% Tween-20
PBS containing 1% BSA (PBS/BSA)
10mg/ml Propidium iodide (PI)
0.1M $Na_2B_4O_7$, pH 8.5

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 CO_2 incubator at 37°C.

2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 minutes, decant supernatant and resuspend in a minimum volume of 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 of 2N HCl containing 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 of 0.1M $Na_2B_4O_7$, 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 10^7/ml.

8. Aliquot 100ul of cell suspension into required number of 12 x 75mm tubes.

9. Incubate the cells with the BrdU antibody at the recommended dilution for 45 minutes at room temperature or overnight at 4°C.

10. Add 2 ml of PBS/BSA and centrifuge the cells at 1000rpm for 5 minutes.

11. If a secondary antibody layer is required then decant the supernatant 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.

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13. Decant the supernatant and add 1ml of PBS containing 10µg/ml PI (Dilute the 10mg/ml solution of PI 1/1000 in a suitable volume of PBS).

14. Analyze cells by flow cytometry following the manufacturer's instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale.

Applications

Table Summary of antibody applications and working conditions

Options / Functions	YES	NO	Not determined	Recommended Work dilution or concentration
ELISA			•	
Western Blotting			•	
IHC – Paraffin			•	
IHC - Frozen			•	
Flow Cytometry (1)	•			Neat - 1/20

Note: Other applications are not tested yet. Optimal dilutions should be determined by each laboratory for each application.
(1) Use 20ul of the suggested working dilution to label 10^6 cells in 100ul. Method sheets are available on request.

Secondary Antibody Applications

Options / Functions	YES	NO	Not determined	Recommended Work dilution or concentration
Immunoassay (ELISA, Western blot)	•			1:5000-1:10000