



## RayBiotech, Inc.

3607 Parkway Lane suite 200  
Norcross, GA 30092  
Tel: 770-729-2992, 1-888-494-8555  
Fax: 770-206-2393  
Website: [www.raybiotech.com](http://www.raybiotech.com)  
Email: [info@raybiotech.com](mailto:info@raybiotech.com)

### Certificate of Analysis and Data Sheet

## Rat Anti-BrdU

With HRP-conjugated Secondary Antibody

**Catalog No.**

DS-MB-00146

**Target Species**

Chemical

**Isotype**

IgG2a

### Synonyms

5- Bromodeoxyuridine

### Preparation

**Purification:** Purified IgG prepared by affinity chromatography on Protein G

### Formulation

**Product Type:** Monoclonal Antibody

**Product Form:** Purified - liquid

**Buffer:** Phosphate buffered saline

**Preservative Stabilizers:** 0.09% Sodium Azide, 25% Glycerol

### Specificity

DS-MB-00146 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°C or at -20°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***

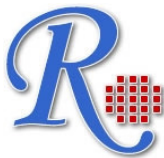
### FLOW CYTOMETRY ANALYSIS

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<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 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<sub>2</sub> 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<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 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<sup>7</sup>/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 10 $\mu$ g/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**

<b>Options</b> <b>Functions</b>	<b>YES</b>	<b>NO</b>	<b>Not determined</b>	<b>Recommended Work dilution or concentration</b>
ELISA			•	
Western Blotting			•	
IHC – Paraffin	•			1/25-1/200
IHC - Frozen			•	
Flow Cytometry	•			1/25-1/200

**Note:** Other applications are not tested yet. Optimal dilutions should be determined by each laboratory for each application.

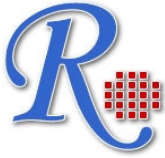
## Secondary Antibody Applications

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

## Reference

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