# *In vivo* BrdU Incorporation and Detection in Murine Plasma Cells

Zheng Liu*

The Feinstein Institute for Medical Research, Manhasset, NY, USA
*For correspondence: zl2119@caa.columbia.edu

**[Abstract]** Bromodeoxyuridine or 5-bromo-2'-deoxyuridine (BrdU) is a synthetic nucleoside that is incorporated into DNA by proliferating cells. This protocol is to be used to incorporate and detect BrdU in murine plasma cells. The plasma cells described in this protocol are formed spontaneously in autoimmune mice (NZB/W mice). Modifications are most likely needed if users intend to label plasma cells in immunized mice.

## Materials and Reagents

A. Antibodies
1. BD mouse Fc Block (BD Biosciences, Pharmingen™, catalog number: 553142)
2. Rat anti-mouse B220 APC (Southern Biotech, catalog number: 1665-11)
3. Rat anti-mouse CD138 PE (BD Biosciences, Pharmingen™, catalog number: 561070)
   *Note: The above antibodies have been tested by the author and may be substituted with the antibodies desired by users.*

B. Other materials
4. Mice
5. FITC BrdU Flow Kit (BD Biosciences, Pharmingen™, catalog number: 559619)
   *Note: *Provided in the kit.*
6. BrdU (Sigma-Aldrich, catalog number: B5002)
7. 1x Dulbecco's modified eagle medium (DMEM) (Life Technologies, Invitrogen™, catalog number: 10313-039)
8. Fetal bovine serum (FBS) (Life Technologies, Invitrogen™, catalog number: 11091-148)
9. Ammonium chloride 10x lysing solution (see Recipes)
10. FACS buffer (see Recipes)

## Equipment

1. BD LSR II flow cytometer

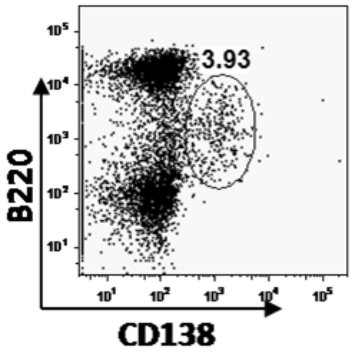
 

**Procedure**

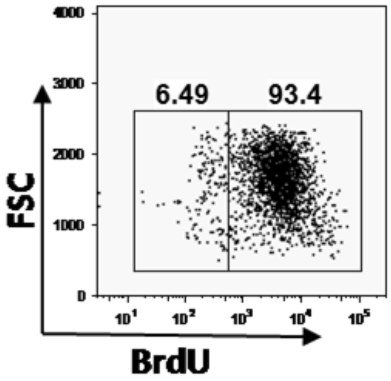
1. Give the mice one i.p. injection of 1 mg BrdU in 200 µl of sterile PBS.
2. Feed the mice water containing 0.8 mg/ml BrdU for 14 days. The water needs to be changed daily and be wrapped in aluminum foil to avoid light.
   *Note: 14 days are needed to label all the newly synthesized plasma cells with BrdU in autoimmune mice (NZB/W mice) and would not result in noticeable toxic effects in the mice.*
3. Sacrifice the mice and harvest the spleen.
4. Create single cell suspension by gently smashing spleen pieces with the frosted surface of a pair of microscope slides in 5 ml of DMEM.
5. Transfer cells into 50 ml conical tubes and spin down cells at 300 *x g* for 5 min at 4 °C.
6. Discard the supernatant with aspiration without disturbing pellet.
7. Resuspend cells with 5 ml of 1x ammonium chloride lysing solution (see Recipes) and incubate on ice for 5 min.
8. Add 15 ml DMEM to cells and spin at 300 *x g* for 5 min at 4 °C.
9. Discard supernatant and resuspend cells with 20 ml of DMEM and count cells.
10. Resuspend 2 millions spleen cells in 50 µl of 1:200 BD Fc block in FACS buffer (see Recipes) and incubate for 30 min on ice.
11. Wash cells with 200 µl of PBS and spin down the cells at 300 *x g* for 5 min at 4 °C.
12. Discard supernatant and resuspend cells in 100 µl FACS buffer containing 1:200 anti-mouse B220 APC and anti-mouse CD138 PE.
13. Incubate cells at room temperature for 15 min.
14. Wash cells as in step 11.
15. Discard supernatant and resuspend cells with 100 µl of BD cytofix/Cytoperm buffer*.
16. Incubate for 30 min on ice for fix cells.
17. Wash cells with 1 ml of BD perm/Wash Buffer* and spin at 300 *x g* for 5 min at 4 °C.
18. Discard supernatant and resuspend cells with 100 µl of BD Cytoperm Plus Buffer*.
19. Incubate cells for 10 min on ice.
20. Wash cells as in step 17.
21. Resuspend cells with 100 µl of BD Cytofix/Cytoperm Buffer*.
22. Incubate for 5 min on ice to re-fix cells.
23. Wash cells as in step 17.
24. Discard supernatant and resuspend cells with 100 µl of diluted DNase* (300 µg/ml in PBS).
25. Incubate cells for 1 h at 37 °C to expose incorporated BrdU.
26. Wash cells as in step 17.

27. Discard supernatant and resuspend cells with 50 µl of BD Perm/Wash Buffer* containing anti-BrdU FITC* (1:50 in buffer).
28. Incubate cells for 20 min at room temperature.
29. Wash cells as in step 17.
30. Resuspend cells in 1 ml of PBS and analyzed stained cells with a flow cytometer (run at a rate no greated than 400 events/second).

    *Note: Cells can be resuspended in 2% formaldehyde and stored overnight at 4 °C in dark, prior to analysis by flow cytometry.*
31. Flow cytometric analysis
    a. Gate on plasma cells (B220 int CD138hi)




    b. Gate on BrdU positive and negative cells




## Recipes

1. Ammonium chloride 10x lysing solution (1 L)

   96 g $NH_4Cl$

   10 g $KHCO_3$

3.7 g $Na_4EDTA$

Add $ddH_2O$ to final volume

Adjust pH to 7.2-7.4 and autoclave

Add $ddH_2O$ 9:1 to make 1x lysing solution

2. FACS buffer

300 µl FBS

10 ml PBS

**Acknowledgments**

This protocol was developed or modified in Dr. Anne Davidson's lab at Feinstein Institute for Medical Research, NY, USA. This work was supported by grants from the NY SLE Foundation (RB), Rheuminations, NIH AI082037 and AR 049938-01, NIH (PO1 AI51392 and the Flow Cytometry and Protein Expression and Tetramer Cores of PO1 AI51392).

**References**

1. Hoyer, B. F., Moser, K., Hauser, A. E., Peddinghaus, A., Voigt, C., Eilat, D., Radbruch, A., Hiepe, F. and Manz, R. A. (2004). Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J Exp Med* 199(11): 1577-1584.
2. Liu, Z., Bethunaickan, R., Huang, W., Lodhi, U., Solano, I., Madaio, M. P. and Davidson, A. (2011). Interferon-alpha accelerates murine systemic lupus erythematosus in a T cell-dependent manner. *Arthritis Rheum* 63(1): 219-229.