BrdU BD kit Intracellular FACS staining

Bromodeoxyuridine (BrdU) (Sigma B5002) is a thymidine analog that is used in cell proliferation studies. BrdU in culture is incorporated into the DNA during DNA synthesis.
BD Pharmingen--> FITC BrdU Flow Kit Ref: 559619, APC BrdU Flow Kit Ref: 552598

1)- Surface staining of the cells in 100ul FACS buffer (PBS, EDTA 5mM, FCS 0.5%) as usually, 30min in on ice.
Spin cells down (1500 rpm / 5min / 4oC). Wash cells with 200ul FACS buffer. Spin them down again.

2)- Resuspend cells in 50ul **Cytofix/Cytoperm** buffer, keep 15-30min at RT or on ice.
Spin cells down. **CAREFULL Perm/Wash buffer is a 10X solution stock !!!!!**

Wash cells with 200ul **Perm/Wash buffer**. Spin them down again.

3)- Resuspend cells in 50ul **Cytoperm Plus** buffer, keep 10min on ice.
Spin cells down. Wash cells with 200ul **Perm/Wash buffer**. Spin them down again.

4)- Resuspend cells in 50ul **Cytofix/Cytoperm** buffer, keep 15-30min at RT or on ice.
Spin cells down. Wash cells with 200ul **Perm/Wash buffer**. Spin them down again.

5)- Resuspend cells in 50ul DNase diluted in **PBS**. (Initial stock solution at 1mg/ml, diluted up to 300ug/ml to have 15ug per sample).
Keep 1h at 37C.

Spin cells down. Wash cells with 200ul **Perm/Wash buffer**. Spin them down again.

NOTE: make a clear negative!!! Cells without BrdU-APC !

Resuspend cells in 50ul **Perm/Wash buffer** containing anti-BrdU-APC diluted at **1/200.**
Keep 20min at RT.
Spin cells down. Wash cells with 200ul **Perm/Wash buffer**! Spin them down again.

7)- Resuspend cells in 100ul FACS buffer, and analyse them with a flow cytometer.

NOTE: BrdU should be stored as a powder in the dark at -20°C in a dessiccator.
Be careful not expose yourself to BrdU dust (this is a mutagen!). Thus to prepare a 10mg/ml solution in PBS, I usually open a new bottle under the fume hood resuspend the totality of the powder at once in sterile PBS and close the bottle immediately. You can prepare a concentrated stock by relying on the information on the label (no need to weigh it on a balance). This way I prepare a solution at 10mg/ml that is then filtered and allicoted in 1ml and kept in the dark at -20°C.

In vivo labeling of mouse cells with BrdU
a) Intraperitoneal Method:
Inject mice i.p. with 100-200 µl (1-2 mg) of a BrdU solution at 10 mg/ml in sterile PBS.
b) Drinking water method:
Dilute BrdU to 0.8 mg/ml in the drinking water. The BrdU-labeled drinking water should be made up freshly and changed frequently due to the exposure to light (preferably every day). Prolonged feeding of BrdU can have toxic effects. Some investigators have reported lethal effects associated with 14 days of continuous BrdU feeding.