

## Datasheet: BUF077A

**BATCH NUMBER 161709**

<b>Description:</b>	CELL STIMULATION REAGENT (WITH BREFELDIN A) (500X)
<b>Name:</b>	CELL STIMULATION REAGENT (WITH BREFELDIN A)
<b>Format:</b>	Reagent
<b>Product Type:</b>	Accessory Reagent
<b>Quantity:</b>	0.1 ml

## Product Details

### Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit [www.bio-rad-antibodies.com/protocols](http://www.bio-rad-antibodies.com/protocols).

	Yes	No	Not Determined	Suggested Dilution
Flow Cytometry	▪			

Where this product has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own system using appropriate negative/positive controls.

**Product Form** 500x concentrate - liquid

**Preparation** 500x solution contains: PMA (phorbol-12-myristate 13-acetate) 40.5 µM, Ionomycin 669.3 µM and Brefeldin A 2.5 mg/ml in DMSO

**Product Information** **Cell Stimulation Reagent (with Brefeldin A) 500x** is a mixture of PMA (phorbol-12-myristate-13-acetate), ionomycin and the protein transport inhibitor Brefeldin A. PMA and ionomycin activate cells and increase cytokine production. Brefeldin A allows for these cytokines to accumulate in the rough endoplasmic reticulum and golgi apparatus allowing for their detection by intracellular fluorescent staining.

**Instructions For Use**

1. Resuspend  $1 \times 10^6$  -  $2 \times 10^6$  cells per ml in cell culture media
2. Thaw the Cell Stimulation Reagent (with Brefeldin A) 500x (BUF077A) in a 37°C water bath
3. To each ml of cell suspension add 2 µl of BUF077A and incubate cells in a CO<sub>2</sub>, 37°C incubator for 6 hours
4. Harvest cells and centrifuge at 300-400 g for 5 minutes. Discard supernatant
5. To wash the pellet, add 10-20 ml of Cell Staining Buffer ([BUF073](#)), vortex to resuspend the pellet then centrifuge at 300-400 g for 5 minutes. Discard supernatant and repeat step

5 for a second time

6. Stain for surface markers or proteins of interest and analyze cells by flow cytometry

Note: Aliquot product to avoid repeat freeze-thawing. Time and culture conditions may need to be optimized.

Activation of cells for >12 hours with Brefeldin A may affect cell viability. In these circumstances it is recommended to use Cell Stimulation Reagent (without Brefeldin A) 500x and then add Brefeldin A Solution 1000x ([BUF075](#)) or Monensin Solution ([BUF074](#)).

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<b>Storage</b>	Store at -70°C. Storage in frost-free freezers is not recommended. This product should be stored undiluted. This product is photosensitive and should be protected from light.
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<b>Guarantee</b>	Guaranteed until date of expiry. Please see product label.
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<b>Health And Safety Information</b>	Material Safety Datasheet documentation #20388 available at: <a href="https://www.bio-rad-antibodies.com/SDS/BUF077A">https://www.bio-rad-antibodies.com/SDS/BUF077A</a> 20388
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<b>Regulatory</b>	For research purposes only
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## Related Products

### Recommended Useful Reagents

[BREFELDIN A SOLUTION \(1000X\) \(BUF075\)](#)

[MONENSIN SOLUTION \(1000X\) \(BUF074\)](#)

**North & South** Tel: +1 800 265 7376

**America** Fax: +1 919 878 3751

Email: [antibody\\_sales\\_us@bio-rad.com](mailto:antibody_sales_us@bio-rad.com)

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To find a batch/lot specific datasheet for this product, please use our online search tool at: [bio-rad-antibodies.com/datasheets](https://www.bio-rad-antibodies.com/datasheets)

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