

Cell Activation Cocktail (without Brefeldin A)

Catalog# / Size	423301 / 100 µL 423302 / 400 µL
Regulatory Status	RUO
Other Names	Cell Stimulation Cocktail, PMA/Ionomycin
Description	Cell Activation Cocktail (without Brefeldin A) is a pre-mixed cocktail with optimized concentration of PMA (phorbol 12-myristate-13-acetate) and ionomycin. Activation of cells by PMA and ionomycin leads to an increase in cell surface expression of certain markers such as CD69 and CD154, which can be detected using this cocktail.

Product Details

Preparation	Cell Activation Cocktail (without Brefeldin A) is composed of PMA and ionomycin.
Concentration	Each vial of this cocktail contains phorbol-12-myristate 13-acetate (40.5 µM) and ionomycin (669.3 µM) in DMSO (500X).
Storage & Handling	Store cocktail at -70°C upon receipt. Protect from light. Avoid repeat freeze/thaw cycles.
Application	Activ, FC, ICFC - Quality tested
Recommended Usage	Cat. No. 423301 contains 100 µL of cocktail, formulated at 500X, that can be used for activating 50 mL of cells of various types. Cat. No. 423302 contains 400 µL (4 vials x 100 µL per vial) of cocktail, formulated at 500X, that can be used for activating 200 mL of cells of various types.

Application Notes	<p>Cell Activation Protocol:</p> <ol style="list-style-type: none"> 1. Resuspend cells of interest in desired cell culture medium. Recommended cell suspension is between 1-2 x 10⁶ cells/mL, although effective activation can be obtained with higher or lower concentration. 2. Completely thaw vials in 37°C water bath. 3. Add 2 µL of the cocktail to each mL of cell suspension. 4. Incubate cells at 37°C in a CO₂ incubator for 6 hours or the time period of interest. 5. Harvest activated cells and centrifuge at 350g for 5 minutes. Discard supernatant. 6. Add 2.5 mL of Cell Staining Buffer (Cat. No. 420201) to the cell pellet, mix by vortex or pipetting, then centrifuge at 350g for 5 minutes. Discard supernatant. 7. Repeat step 6. 8. Cells are now ready to be tested for surface markers or proteins of interest.
--------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Note: If investigators are interested in detecting intracellular proteins following activation, this product can be used in conjunction with Monensin (Cat. No. 420701) or Brefeldin A (Cat. No. 420601).

This product is temperature sensitive; repeated freeze/thaw is not recommended.

Intracellular and surface flow staining, as well as ELISA, can be performed following activation of cells with this cocktail.

Additional Product Notes	View more applications data for this product in our Scientific Poster Library .
---------------------------------	-------------------------------------------------------------------------------------------------

Product Citations	<ol style="list-style-type: none"> 1. Chen J, <i>et al.</i> 2022. Adv Healthc Mater. 11:e2101714. PubMed 2. Matiza T, <i>et al.</i> 2021. Medicine (Baltimore). 100:e28328. PubMed 3. Cella M, <i>et al.</i> 2019. Nat Immunol. 1.513888889. PubMed 4. Carpenter RS, <i>et al.</i> 2019. Sci Rep. 9:19105. PubMed 5. McLean BA, <i>et al.</i> 2021. JCI Insight. 6:. PubMed 6. Ayenehdeh J, <i>et al.</i> 2017. Immunology Letters. 10.1016/j.imlet.2017.05.006. PubMed 7. Vazquez J, <i>et al.</i> 2020. Front Immunol. 2.545138889. PubMed 8. Nagle VL, <i>et al.</i> 2022. Mol Cancer Ther. 21:658. PubMed 9. Cai B, <i>et al.</i> 2021. Mol Cancer. 20:165. PubMed 10. Glaubitz J, <i>et al.</i> 2022. Nat Commun. 13:4502. PubMed 11. Datta A, <i>et al.</i> 2022. J Fungi (Basel). 8:. PubMed 12. Stephenson E, <i>et al.</i> 2021. Nat Med. 27:904. PubMed
--------------------------	-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

13. Qi X, *et al.* 2019. *Nat Med.* 25:1225. [PubMed](#)
14. Bunting MD, *et al.* 2022. *Sci Adv.* 8:eabk3327. [PubMed](#)
15. Uddin MN, *et al.* 2022. *Brain Behav Immun Health.* 26:100563. [PubMed](#)
16. Lee J, *et al.* 2020. *Sci Rep.* 10:17753. [PubMed](#)
17. Owyong M, *et al.* 2019. *Life Sci Alliance.* 2:e201800226. [PubMed](#)
18. Kumar RK, *et al.* 2021. *Vascul Pharmacol.* 138:106838. [PubMed](#)
19. Huang RS, *et al.* 2021. *Curr Protoc.* 1:e246. [PubMed](#)
20. Chen YF, *et al.* 2019. *J Biomed Sci.* 26:85. [PubMed](#)
21. Tajima M, *et al.* 2022. *Curr Protoc.* 2:e540. [PubMed](#)
22. de Picciotto S, *et al.* 2022. *Nat Commun.* 13:3866. [PubMed](#)
23. Einkauf KB, *et al.* 2022. *Cell.* 185:266. [PubMed](#)
24. Teh MR, *et al.* 2021. *Front Immunol.* 12:714613. [PubMed](#)
25. Michoud F, *et al.* 2020. *Nat Biotechnol.* . [PubMed](#)
26. FitzPatrick MEB, *et al.* 2021. *Cell Rep.* 34:108661. [PubMed](#)
27. Trial J, *et al.* 2020. *Geroscience.* . [PubMed](#)
28. Spencer AJ, *et al.* 2022. *EBioMedicine.* 77:103902. [PubMed](#)
29. Wang F, *et al.* 2021. *Neoplasia.* 23:281. [PubMed](#)
30. Mysore V, *et al.* 2021. *Med (N Y).* 2:1050. [PubMed](#)

Antigen Details

Gene ID NA

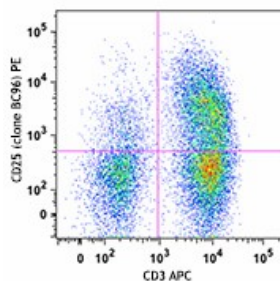
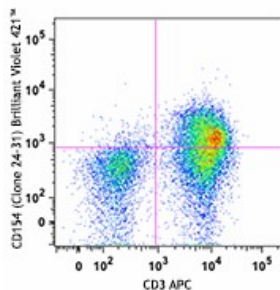
Related Protocols

[Surface and Intracellular Cytokine Staining for Flow Cytometry - Video](#)

[Cell Surface Flow Cytometry Staining Protocol](#)

[Intracellular Flow Cytometry Staining Protocol](#)

Product Data



Human peripheral blood lymphocytes were stimulated with Cell Activation cocktail without Brefeldin A for six hours, then surface stained with CD3 APC and CD154 (clone 24-31) Brilliant Violet 421™ (top) or CD25 (clone BC96) PE (bottom).

For research use only. Not for diagnostic use. Not for resale. BioLegend will not be held responsible for patent infringement or other violations that may occur with the use of our products.

*These products may be covered by one or more Limited Use Label Licenses (see the BioLegend Catalog or our website, www.biolegend.com/ordering#license). BioLegend products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products, reverse engineer functionally similar materials, or to provide a service to third parties without written approval of BioLegend. By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic,

therapeutic or commercial use.

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587