Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Non WHO Reference Material
ICS positive control cells
NIBSC code: 15/272
Instructions for use
(Version 1.02, Dated 27/07/2021)

This material is not for in vitro diagnostic use.

1. INTENDED USE

The material contains stabilised, stimulated cells that are intended for use as a positve control for intracellular cytokine staining methods and enumeration by flowcytometry. The material can be used to validate changes in equipment, operator or protocol for inter and intra-laboratory performance monitoring and training and qualifying new users or establishing new assays. The product is not intended for use as an in vitro diagnostic device (IVD). The reference material consists of stabilised pooled peripheral blood mononuclear cells stimulated with PMA/ionomycin for 4-6h in the presence of a protein transport inhibitor. Each reconstituted vial contains stimulated T cells, that produce IL-2, IFN-gamma and TNF-alpha.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The reagent has no assigned unitage and is not intended for use as a calibrator. The mean cytokine positive values for IL-2, IFN-gamma and TNF-alpha are stated in section 4.

4. CONTENTS

Country of origin of biological material: United Kingdom.

The material is a stabilised, lyophilised human peripheral blood mononuclear cell preparation, made from pooled donations obtained from the UK National Blood Service. Isolated cells are stimulated in vitro with PMA/ionomycin in the presence of brefeldin A (Rajagopal et. al., 2020). The material has been characterised in house and shows a mean +/- SD value of 69.9 +/- 0.8% and 25.4 +/- 0.4%; CD4 and CD8-positive T cells respectively. CD4 T cells positive for intracellular cytokines, IL-2, IFN-gamma and TNF-alpha show mean +/- SD value of 34.3 +/- 0.5%, 7.2 +/- 0.2% and 22.7 +/- 1.1% respectively. CD8 T cells positive for intracellular cytokines, IL-2, IFN-gamma and TNF-alpha returned an overall mean +/- SD of 19 +/- 0.9%, 29.4 +/- 1.4% and 30.4 +/- 1.3% respectively. Mean values were obtained from in house repeat testing of reference material. Figure 1 shows the representative gating strategy.

5. STORAGE

Reference materials are manufactured and held at NIBSC within assured, temperature-controlled storage facilities. On receipt, reference Materials should be stored below -20°C until use.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Reconstitute the material by dissolving entire contents of the vial in 1ml sterile distilled water at room temperature and allow it to equilibrate for 10-30 min before use. 0.1 ml of the reconstituted cell suspension is stained for surface markers (CD3, CD4 and CD8) for 40 min in dark at room temperature. Cells are permeabilized and then stained for intracellular cytokines, IL-2, IFN-gamma and TNF-alpha. Mean values for intracellular cytokine positive cells was determined using the above staining method. The extent to which further dilutions allow for in range values has not been assessed and is the responsibility of the end user.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

An accurate and rapid single step protocol for enumeration of cytokine positive T lymphocytes. Rajagopal D, Tian L, Xiong S, Wang L, Campbell J, Saraiva L, Vessillier S. Journal of Immunology and Regenerative Medicine (2020). 9: 100032

10. ACKNOWLEDGEMENTS

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	No
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Contains material of human origin		

Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin

Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Wash mouth with water. Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.

Action on Spillage and Method of Disposal
Spillage of vial contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.5 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No

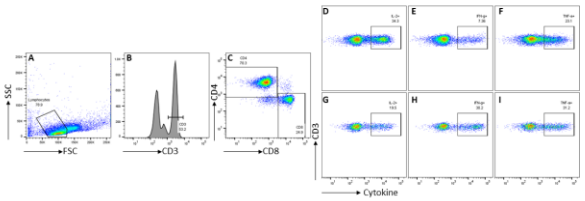

Figure 1: Representative gating strategy and FACS plots for analysis of 15/272 reference material: Cellular reference material, 15/272 (PMA/ionomycin stimulated PBMC) was stained for surface markers with antibodies for CD3, CD4 and CD8 and subsequently stained for intracellular cytokine following permeabilization. Cells were gated based on forward scatter versus side scatter to identify lymphocyte population (1A). Lymphocytes were subsequently gated for $CD3^+$ cells (1B). $CD4^+$ and $CD8^+$ T cells were next gated as subsets of $CD3^+$ T cells (1C). IL-2 (1D, 1G), IFN-γ (1E, 1H) and TNF-α (1F, 1I) positive gate was set based on staining with respective isotype control for each cytokine. Panels 1D-1F show intracellular cytokine staining on $CD4^+$ T cells and panels 1G-1I show intracellular cytokine staining on $CD8^+$ T cells.

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