Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
1st WHO International Reference Reagent
CD4 T-cells (human)
NIBSC code: 15/270
Instructions for use
(Version 1.0, Dated 10/12/2018)

1. INTENDED USE

This material is intended for use as a cellular control for CD4 T cell enumeration by flow cytometry. It has been qualified using single-platform and dual-platform classical flow cytometers, and the point of care device InstantCount. The material can be used to compare different technologies for CD4 T cell counting, to validate changes in equipment, operator or protocol, for inter- and intra-laboratory performance monitoring, and for training and qualifying new users or new assays. Each reconstituted vial contains unlabelled stabilised human leukocytes, evaluated for CD4 T cells per microliter and percentage CD4 T cells by expert laboratories.

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

This reagent has no assigned unitage and is not intended to be used as a calibrator. The mean cell count and range obtained in the collaborative study are stated in section 4.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The material is stabilised lyophilised human blood leukocytes pooled from donations to the UK National Blood Service.
In the hands of expert laboratories, material 15/270 returned an overall mean of 336 CD4 T cells/µL, with an intra-laboratory CV between 4 and 6% for most laboratories and a maximum intra-laboratory CV of 16%. The mean value obtained by an individual laboratory upon repeat testing is expected to fit within the range of 272-400 CD4 T cells/µL with a maximum CV of 16%.

Expert laboratories returned a mean %CD4 among leukocytes of 46%. Percentage CD4 values are expected to be between 40 and 52%.

5. STORAGE

Store unopened ampoules at -20°C or below.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Take ampoule out of storage, place in a tube holder and allow to adjust to room temperature. Break ampoule seal. RECONSTITUTE CONTENTS OF THE AMPOULE WITH 1mL OF STERILE DISTILLED WATER.
Allow 5-30 min for rehydration. Mix cell suspension well and transfer to a capped tube. Process the material as a normal patient sample. Red blood. cell lysis reagents are not required. One commercial brand of red blood cell lysis buffer tested resulted in inadequate discrimination of CD4+ and CD4- lymphocytes. This effect was not seen with other brands of red blood cell lysis buffer tested, in-house ammonium chloride lysis buffer or phosphate buffered saline. For this reason, users need to validate the use of their lysis buffer. Please contact us using the Customer Feedback email if you require information on red blood cell lysis buffer compatibility.

The material has been qualified in single and dual-platform classical flow cytometers and the point of care device InstantCount. There is the possibility of lack of interpretable results using other assay systems.

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.

For short term storage up to 2 days transfer the reconstituted material in a capped tube to 4°C. For longer storage, users should determine the stability of the reconstituted material according to their own storage facilities.
NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

1. Saraiva, LJ, Sutherland, J; Stebbings, R; Hockley, J; Rigby, P; Vessillier, S. An international collaborative study to establish a WHO international reference reagent for CD4 T cell counting. 2018: ECBS report (http://www.who.int/biologicals/BS.2018.2333_CD4_Cell-Counting.pdf?ua=1)

2. World health organization: Summary of the study protocol for multicentre evaluation of CD4 technologies as part of the WHO pre-qualification of diagnostics programme (PQDx). 2017. (http://www.who.int/diagnostics_laboratory/evaluations/cd4/en/)

10. ACKNOWLEDGEMENTS

We are deeply thankful to the participants in this collaborative study who have dedicated their time to the project.

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

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

World Health Organization

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

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:	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 biological 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: 1.0g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use.

The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

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