



**WHO Reference Reagent
RHD/SRY Plasma DNA sensitivity standard
NIBSC code: 07/222
Instructions for use
(Version 3.0, Dated 23/05/2014)**

1. INTENDED USE

The material 07/222 is RhD positive male plasma diluted in an excess of RhD negative female plasma. This material was established in 2010 as the 1st WHO Reference Reagent, RHD/SRY Plasma DNA sensitivity standard, by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO).

When reconstituted and diluted as described below, it should be used as a reference reagent for minimum acceptable potency for the detection of RHD and SRY sequences in cell-free plasma DNA. This material should not be put to any other use.

2. CAUTION

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

The preparation contains material of human origin, which has been tested and found negative for antibodies against HIV1, HCV, and HBV. PCR tests for HCV and HIV on both plasmas were also negative. 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

No units are assigned to this material.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1 ml pooled human plasma. The plasma was collected from two donors and anticoagulated with citrate.

5. STORAGE

Store all unopened ampoules of the freeze-dried preparations 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

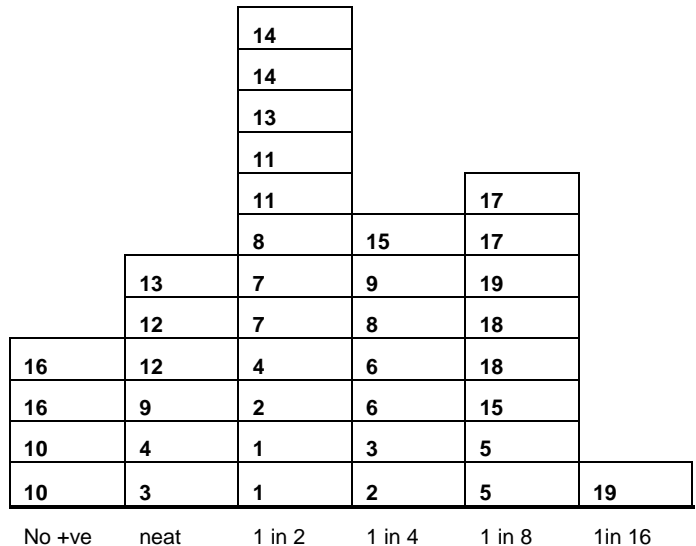
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

The ampoules do not contain bacteriostat and the preparation should not be assumed to be sterile. Each ampoule should be reconstituted with 1.0 ml nuclease-free water and allowed to stand for 5 minutes with occasional gentle mixing before the DNA extraction procedure is started.

After DNA extraction, dilute the DNA by adding an equivalent volume of nuclease-free water. Diluted material should then be tested for the presence of RHD and SRY DNA sequences. This dilution (1 in 2) is the minimum dilution expected to be detectable in real-time PCR assays.

However, many laboratories can detect the targets at higher dilutions, as shown in the following histogram which is taken from the collaborative study report.

Figure 1. Dilution series from WHO Collaborative Study, last dilution reported as RHD gene present. Two ampoules tested per lab. Numbers in boxes indicate laboratory code number. No +ve: no overall RHD positive conclusions for any dilution.



8. STABILITY

Reference materials are held at NIBSC within assured, temperature controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. Accelerated degradation studies have indicated that this material is suitably stable, when stored at -20°C or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned value.

9. REFERENCES

P Metcalfe, P Rigsby, E Tait, S Urbaniak. An International Reference Reagent for the detection of RHD and SRY DNA in plasma. *Vox Sanguinis* 2012, 102, 243-249.

10. ACKNOWLEDGEMENTS

We would like to thank SNBTS Aberdeen for supplying materials and assistance with the collaborative study.

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



Attached: No

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

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 solid	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 ampoule 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: 0.0703g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.

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_biol_efstandardsrev2004.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.