Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

International Ref. Reagent
Mullerian Inhibiting Substance/Anti-Mullerian Hormone, human, recombinant
NIBSC code: 16/190
Instructions for use
(Version 1.0, Dated 20/11/2019)

1. INTENDED USE

This reference reagent (coded 16/190) consists of a batch of lyophilized ampoules containing purified, recombinant human Mullerian Inhibiting Substance, also known as Anti-Mullerian Hormone (AMH). The preparation was established as the 1st WHO International Reference Reagent at the 70th meeting of the WHO Expert Committee on Biological Standardisation (2019). The intended use is for the characterization of AMH assays.

2. CAUTION

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

The material is of bovine origin. The material is certified to be obtained from animals taken from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having BSE & which has not been fed rations containing ruminant derived protein during that period. 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

By collaborative study, immunoassay estimates provided a robust geometric mean content estimate of 489 ng per ampoule.

4. CONTENTS

Country of origin of biological material: USA.
Each ampoule contains:
Recombinant human AMH
2.5 mg trehalose
1.2 mg bovine casein

5. STORAGE

Unopened ampoules should be stored at -20ºC
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
For all practical purposes, each ampoule contains the same quantitiy of the substances listed above. The material has not been sterilized and the ampoules contain no bacteriostat. Depending on the intended use, dissolve the total contents of the ampoule in a known volume of a suitable diluent. Users should make their own investigations into the type of diluent suitable for their use. If extensive dilutions are prepared, a carrier protein should be added.

PREPARATION OF AMPOULES AND COLLABORATIVE STUDY
Recombinant AMH was purified from the culture media of a stable CHO cell line, LR-MIS, expressing the native human AMH sequence with the leader sequence from human serum albumin (HSA) and with a modification of the internal cleavage site at amino acids 423-428 from RAQR/S to RARR/S [1]. The HSA leader sequence is cleaved during maturation. A 2100 ml volume of bulk formulation containing 0.24% (w/v) casein (Calbiochem), 0.5% (w/v) trehalose (Sigma) and nominally 1 µg/ml AMH was distributed into 3 mL siliconized DIN ampoules as 0.5 ml aliquots. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen.

The batch of ampoules, coded 16/190, was evaluated in a collaborative study by immunoassays performed by seven laboratories in four countries providing 21 data sets, 16 of which met the assay validity criteria of slope [0.91, 1.10] and ratio of coded duplicates [0.91, 1.10]. Reported estimates of content in terms of method calibrations gave a geometric mean estimate of 511 ng/amp (95% CI: 426-612 ng/amp, n=16, GCV 42.0%) and a robust geometric mean of 489 ng/amp. Commutability was assessed by the concomitant measurement of 22 patient samples. By the difference in bias approach, dilutions of 16/190 were shown to be fully within the statistically-defined limits of commutability for 6 of the 16 methods. Of the remaining methods, some dilutions of 16/190 were within the limits for 3/16 methods whereas no dilutions were within the defined limits for 7/16 methods [2]. Manufacturers are recommended to perform an assessment of the commutability of 16/190 with patient samples when measured by their method.

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. thus, no expring date is assigned to international reference materials. Analysis of acclerated thermal degradation samples stored for 21 months and measured by participants in the collaborative study showed no significant loss of AMH immunoreactivity suggesting that 16/190 is likely to be highly stable when stored at -20ºC. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

[1] Pépin, D et al., (2013) An albumin leader sequence coupled with a cleavage site modification enhances the yield of recombinant C-terminal Mullerian Inhibiting Substance. Technology 2013; 1, 63-71.
[2] Ferguson, J. et al., (2019) WHO International Collaborative Study of the Proposed WHO IS for Mullerian Inhibiting Substance/Anti-Mullerian Hormone, human, recombinant.
https://www.who.int/biologicals/expert_committee/BS.2019.2363_Anti-Mullerian_Hormone_FINAL.pdf

10. ACKNOWLEDGEMENTS

We gratefully acknowledge Professor Patricia Donahoe (Director) and Dr David Pépin of the Pediatric Surgical Research Laboratories, Massachusetts General Hospital for the donation of the bulk material, the important contributions of all the participants in the collaborative study, the Standardization Science Group at NIBSC for preparation of trial materials, the Standards Processing Division at NIBSC for the preparation and dispatch of the ampouled materials and the Biostatistics Group at NIBSC for analysis of the collaborative study data

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: 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

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

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):			

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.

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

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: 0.003g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No