ANTI-H

Anti-H Lectin (*Ulex europaeus*)

For Tube Test

Cat No.	Size
1/136	2ml
1/140	5ml
1/140d	10ml

INTRODUCTION

Intended Use

BIOTEC Anti-H (lectin) is used to demonstrate the presence of H precursor on human red cells and can be used in ABH secretor status determination. A tube technique should be used with this reagent. For professional *in vitro* diagnostic use only.

Determination of Secretor Status

Approximately 80% of the population have soluble ABO blood group antigens present in their body fluids. These patients are known as 'ABH Secretors', while the remaining 20% are 'Non-Secretors'. The ability to secrete these soluble antigens is controlled by a pair of alleles, *Se* and *se*, at the secretor locus. *Se* is the gene responsible for the secretion of these soluble antigens. Secretors are *Se/Se* or *Se/se* while non-secretors are *se/se*.

There is direct link between the ability to secrete ABH substances and the Lewis blood groups, due to the ability or inability to produce ά-2-fucosyltransferase. ABH non-secretors have no ά-2-fucosyltransferase to convert resulting in the presence of the Le(a+b-) Lewis blood group, while secretors will have the Lewis blood group Le(a-b+). The Lewis phenotype Le(a-b-) can be found in secretors and non-secretors.

The ability to secrete or the inability to secrete soluble ABH antigens has no known effect on the general health of the individual although it may be a useful tool in the hands of the forensic scientist.

Secretors contain the following ABH substances in their body fluids:

O secretor	H only
A secretor	A and H
B secretor	B and H
AB secretor	A, B and H
Non secretor	None

Principle of the Method

The test procedure is based on the principle of agglutination.

PRODUCT CONTENTS

BIOTEC anti-H (lectin) is an extract from seeds of *Ulex europaeus* in a buffered medium. The reagent contains 0.09% sodium azide as a preservative and is provided ready for use.

ITEMS REQUIRED BUT NOT PROVIDED

- Phosphate buffered saline (PBS) pH 6.8-7.2
- Centrifuge (1000rpm)
- Glass test tubes
- Glass reaction slide
- Control ABO group O red cells (positive control)
- Control A_1 red cells (negative control)
- Anti-A and Anti-B reagents (secretor status determination only)

STORAGE AND SHELF LIFE

Store at 2-8°C. This reagent is stable until the expiry date given on the label, when stored as directed.

WARNINGS AND PRECAUTIONS

- This reagent must not be diluted. A balance of buffers enhances the reaction and further dilution will seriously damage its ability to agglutinate red cells.
- *Ulex* extract has often been shown to be an unstable reagent. Consequently known ABO group O cells should always be included in any batch of tests to give the known positive reaction.
- Only hh (Bombay) red cells will give a true negative reaction with this reagent.
- The reagent occasionally shows slight turbidity after storage. This is usually due to the aggregation of solids at 2-8°C and appears light brown. A white deposit is usually crystals from the buffering salts. Neither of these deposits will interfere with the agglutinating ability of the reagent.
- Follow local guidelines for waste disposal. A material safety data sheet (MSDS) is available on request.

SPECIMEN PREPARATION

Blood

Draw a blood specimen using an acceptable phlebotomy technique.

Saliva

For determination of secretor status.

Saliva specimens should be collected after rinsing the patient's mouth thoroughly with water to remove cells and other particles.

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ANTI-H

Collect a few millilitres of saliva in a glass tube and boil for 5 minutes to destroy enzymes. The saliva will consist mainly of water so an equal volume of saline should be added to ensure that the red cells are not haemolysed. Centrifuge at 1000rpm for 1 minute and draw off the clear supernatant for testing. Samples may be frozen if testing is proposed at a later date.

BLOOD GROUPING STUDIES PROCEDURE

1. Wash the red cells to be tested four times in PBS pH 6.8-7.2. Resuspend to a 5% cell suspension in PBS.
2. Place in a glass test tube (50 x 7mm):
   - 1 drop BIOTEC antisera
   - an equal volume of 5% cell suspension
3. Mix well and incubate for 5-10 minutes at room temperature.
4. Centrifuge at 1000rpm for 1 minute.
5. Pipette sedimented cells to a glass slide and read agglutination.

INTERPRETATION

Cells	Result
A_1	Will show weak or no agglutination
A_2	Will show a weak to + reaction
B	Will show a + to ++ reaction
O	Will show a strong +++ to ++++ reaction

Quality Control

Control ABO group O red cells should be used as a positive control and group A_1 as a weak/negative control with each batch of tests.

DETERMINATION OF SECRETOR STATUS PROCEDURE

Notes:

- The user should ensure that only *Ulex europaeus* extract is used since it has been shown that other lectin extracts can produce erroneous results [(1)].
- It will be necessary to dilute the Anti-A and the Anti-B to a point where only a one plus reaction is obtained against the appropriate red cells.
- The Anti-H can be used straight from the bottle without dilution.
- The test principle is that of agglutination inhibition.
- This recommended procedure gives only the general principles used for the determination of secretor status in the individual. The subject is complex and the reader is advised to read further [(2)] where there is clinical application for the tests to be undertaken.

Method

1. Place in a glass test tube (75 x 12mm):
   - 1 drop of BIOTEC antisera (Anti-A, -B or -H)
   - an equal volume of saliva
2. Incubate for 5 minutes at room temperature. This will allow any substance in the saliva to neutralise the antisera.
3. Add 1 drop of the appropriate red cells (A, B or O).
4. Incubate for 5 minutes at room temperature.
5. Centrifuge at 1000rpm for 1 minute and read for agglutination.

Results

Agglutination of the appropriate red cells indicates that the saliva did not contain any ABH soluble antigens and indicates that the individual is a non-secretor.

Inhibition of the agglutination indicates that soluble antigens in the saliva neutralised the antisera indicating that the individual is a secretor.

	Anti-A	Anti-B	Anti-H
O secretor	+++	+++	-
A secretor	-	+++	-
B secretor	+++	-	-
AB secretor	-	-	-
O non-secretor	+++	+++	++
A non-secretor	+++	+++	++
B non-secretor	+++	+++	++
AB non-secretor	+++	+++	++

REFERENCES

1. Race RR, Sanger R. *Blood Groups in Man*, 6th edn. Oxford: Blackwell Scientific Publications 1975.
2. Daniels G. *Human Blood Groups,* 1st. edn. Blackwell Science Ltd. 1995.

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