



# ATLAS Anti – D Clone 1 & Clone 2 For Tube, Slide, Microplate and DiaMed – ID Techniques

For *In-Vitro* and professional use only  
Store at 2° to 8° C

## SUMMARY

The Rh blood group system was discovered in 1940. Rh D negative. The D antigen is the most clinically significant non – ABO red blood cell antigen and has been implicated in causing Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

		Prevalence%	
Anti – D	Phenotype	Caucasians	Afro – Americans
+	Rh D +ve	85	72
0	Rh D –ve	15	28

## PRINCIPLE

When used by the recommended techniques, the reagent will cause direct agglutination (clumping) of red cells carrying corresponding specific antigen. No agglutination usually indicates the absence of the corresponding specific antigen (See Limitations)

## REAGENT

Atlas Monoclonal IgM Anti – D is a low protein reagent containing a human monoclonal IgM antibody diluted with sodium chloride, bovine albumin and macromolecular potentiators. The reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D<sup>V1</sup>) and a high proportion of weak D (D<sup>w</sup>) phenotypes. The reagent is supplied at optimal dilution for use with all recommended techniques without need for further dilution or addition. Lot reference number and expiry date are printed on the individual vial labels.

Product	Cell Line/Clone
Clone 1	RS – 1126
Clone 2	MS - 201

## STORAGE CONDITIONS, TRANSPORTATION & HANDLING

For transportation overseas this product will remain stable for up to 7 days when subjected to temperatures not exceeding 30°C. Upon arrival store all vials at 2 - 8°C. Do not freeze or expose to elevated temperatures. Prolonged storage outside the recommended temperature range may result in accelerated loss of reagent reactivity. Protective clothing should be worn when handling the reagent, such as disposable gloves and a laboratory coat

## SPECIMEN COLLECTION

Specimens may be drawn with or without anticoagulant, using an aseptic phlebotomy technique. If testing is delayed then store should be tested within 48 hours of being drawn. Clotted specimens may be tested up to 14 days from date drawn. Specimens collected into ACD, CPD, or CPDA – 1 can be tested up to their expiry date.

## PRECAUTIONS

- The reagent is intended for in vitro diagnostic use only.
- If vial is cracked or leaking, discard the contents immediately.
- Do not use reagent past the expiration date (see Vial Label).
- Do not use reagent if a precipitate is present.
- The reagent has been sterile filtered through a 0.2 µm capsule to reduce bio – burden. Once vial has been opened the contents should remain viable up until expiry date as long as there is no marked turbidity indicating reagent contamination.
- The reagent contains 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
- Materials used to produce the product were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HbsAg using approved microbiological tests. No known tests can guarantee products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

## CONTROLS AND ADVICE.

- It is recommended positive (R<sub>1r</sub>) and negative (rr) control red cells be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not shown expected results.
- When typing red cells from a patient suspected of having autoantibodies or protein abnormalities a reagent control is needed.
- In **Recommended Techniques** one volume is approximately 40 µl when using the vial dropper provided.
- Use of reagent and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagent is in use.
- The user must determine the suitability of the reagent for use in other techniques.

## MATERIALS REQUIRED:

### A. Reagent Supplied:

- Atlas Monoclonal Anti – D Clone 1 Blood Grouping Reagent.
- Atlas Monoclonal Anti – D Clone 2 Blood Grouping Reagent

### B. Materials And EQUIPMENT Not Supplied:

- Glass test tubes (10x75mm or 12x75mm).
- Pasteur pipettes.
- Centrifuge (for tube and microplate tests).
- Glass slides.
- Applicator sticks.
- Validated “U” well microplates.
- Plate shaker and automatic plate reader.
- DiaMed ID – Cards (neutral).
- DiaMed ID – Centrifuge with fixed rpm and time.

### B. Additional Reagents Required:

- Phosphate Buffered Saline (PBS): Containing 8.5 to 9.0g/l NaCl (0.145M – 0.154M) pH 7.0 ± 0.2 at 22°C ± 1°C.
- DiaMed ID – Diluent: e.g. ID – CellStab.
- Known positive (R<sub>1r</sub> cells) and negative (rr cells) controls.
- Known Negative Control for Monoclonal Anti – D reagents

## RECOMMENDED TECHNIQUES:

### A. Tube Technique

1. Prepare a 2 – 3% suspension of test red cells in PBS.
2. Place in a labeled test tube: 1 volume of Atlas Anti – D and 1 volume of test red cell suspension.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend each cell button and read macroscopically for agglutination.
5. Any tubes which show a negative or questionable test result should be incubated for 15 minutes at 18 - 25°C.
6. Following incubation, repeat steps 3 and 4.

### B. Slide Technique

1. Prepare a 35 – 45% suspension of test red cells in autologous serum or plasma.
2. Place on a labeled glass slide: 1 volume of Atlas Anti – D and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix the reagent and cells over an area of about 20x40mm.
4. Slowly tilt slide back and for 30 seconds, with occasional further mixing during 2- minute period.
5. Read macroscopically after 2 minutes over a diffuse light, to not mistake fibrin strands as agglutination .
6. Any weak reaction should be repeated by the tube technique.

### C. Microplate Technique Using “U” Wells

1. Prepare a 2-3 % suspension of test red cells in PBS.
2. place in the appropriate well: 1 volume of Atlas Anti –D and 1 volume of test red cell suspension .
3. Mix well , preferably with a microplate shaker , taking care to avoid cross –well contamination .
4. Incubate at 18-25°C for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker.

7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique

### D. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of red cells in DiaMed ID- Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50µl of test red cell suspension and 25 pl of Atlas Anti –D.
4. Centrifuge the ID-card for 10 minutes in an ID-centrifuge.
5. Read macroscopically for agglutination.

### INTERPRETATION OF RESULT TS:

1. **POSITIVE:** Agglutination of red cells after centrifugation constitutes a positive test result and within the accepted limitations of the test procedure , indicates the presence of the Rh D antigen on the red cells.
2. **Negative :**no agglutination of red cells after centrifugation constitutes a negative result and within the accepted limitations of the test procedure ,indicates the absence of the Rh D antigen on the red cells.

### STABILTY OF THE REACTIONS:

1. Read all tube and microplate tests straight after centrifugation .
2. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent .
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those mentioned in the **Recommended Techniques**.

### LIMIATIONS :

1. Atlas Anti-D is not suitable for use with enzyme treated cells or for use in indirect antiglobulin techniques .
2. Stored blood may give weaker reactions than fresh blood.

3. False positive or false negative results may occur due to:
  - Continuation of test materials
  - Improper cell concentration
  - Improper incubation time or temperature
  - Improper or excessive centrifugation
  - Improper storage of test material or omission of reagents
  - Deviation from the recommended techniques

### SPECIFIC PERFORMANCE CHARACTERISTICS:

1. The reagent has been characterized by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, red cells to ensure suitable reactivity.
3. The potency of the reagent is tested against the following minimum potency reference standard obtained from, National Institute of Biological Standards and Controls (NIBSC):
  - Anti – D reference 91/592.
4. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen – negative cells.
5. The Quality Control of this reagent was performed using red cells that had been washed twice with PBS prior to use.
6. The latest issue of the Guidelines for the UK Blood Transfusion Services, Section 3.

### DISCLAIMER:

1. The user is responsible for the performance of the reagent by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations should be validated prior to use as specified in the article: Recommendations for evaluation and implementation of new techniques for blood grouping, antibody screening and cross matching.

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