	REFERENCE	CLONE
ANTI-Cw (RH8)	78013	MS110
ANTI-Fyb (FY2)	78015	SpA264LBg1
ANTI-Jka (JK1)	78003	P3HT7
ANTI-Jkb (JK2)	78004	P3 143
ANTI-Lea (LE1)	78009	13643B9
ANTI-Leb (LE2)	78010	GX336
ANTI-M (MNS1)	78005	2514E6
ANTI-N (MNS2)	78006	1422C7
ANTI-P1	78011	650

DIAGAST

INTRODUCTION

These reagents are *in vitro* diagnostic medical devices (IVDMD) for professional use. They are intended for the analysis of human biological specimens. They are used in the phenotyping of human red blood cells. The reagents are designed to determine the presence of antigens C^w (RH8), Fy^b (FY2), Jk^a (JK1), Jk^b (JK2), Le^a (LE1), Le^b (LE2), M (MNS1), N (MNS2) and P1 on the surface of red blood cells.

PRINCIPLE

The manual tube method used is based on the principle of haemagglutination. Red blood cells bearing an antigen agglutinate in the presence of the reagent containing the corresponding antibody:

- either using the direct haemagglutination method,
- or using papain.

Papain, a proteolytic enzyme derived from the papaya (*Carica papaya)* induces a marked decrease in the electronegative charge on the surface of red blood cells, enabling their agglutination by normally 'non-agglutinating' antibodies in saline medium.

COMPOSITION

The reagents are produced by DIAGAST from red cell monoclonal antibodies and presented in a storage medium.

The monoclonal antibodies ANTI-Cw, ANTI-Fyb, ANTI-Jka and ANTI-Jkb of IGM type, are derived from the *in vitro* culture supernatant of hybridomas of human origin.
The monoclonal antibodies ANTI-Lea, ANTI-Leb and ANTI-P1 of IGM type, are derived from the *in vitro* culture supernatant of hybridomas of murine origin.
The monoclonal antibodies ANTI-M and ANTI-N of IgG type are derived from the *in vitro* culture supernatant of hybridomas of murine origin.

All the reagents contain sodium azide (< 0.1%). All the reagents except ANTI-Cw (RH8), ANTI-P1 and ANTI-Fyb (FY2) contain sodium arsenite (0.02 %). All the reagents except ANTI-Jkb (JK2) contain bovine albumin.
ANTI-Fyb (FY2) contains Proclin at 0.05%.
The reagents are packaged in 3-mL vials fitted with calibrated droppers.

PRECAUTIONS

WARNING (Only for ANTI-Fyb (FY2) containing Proclin at 0.05%)

Particulars of danger **:**
H317 May cause an allergic skin reaction.
Advice of caution **:**
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P272 Contaminated work clothing should not be allowed out of the workplace.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P302+P352 IF ON SKIN: Wash with plenty of water.
P501 Dispose of contents/container in accordance with the local regulations, regional, national and international.

It is advisable to wear gloves and safety spectacles and handle samples of human origin with caution. All materials that have come into contact with the samples are to be handled as potentially infectious products. Special protective measures and conditions for disposal and disinfection should be implemented in accordance with local regulations. Do not use damaged or leaking reagents.

STORAGE

All the reagents except ANTI-Lea (LE1) antibody are to be stored between +2ºC and +8ºC.
ANTI-Lea (LE1) antibody is to be stored between –18ºC and –30ºC. Do not refreeze after thawing. After thawing, ANTI-Lea (LE1) can be stored between +2ºC and +8ºC for up to 6 months.
The performance of these reagents is guaranteed for the recommended methods from first use to the expiry date indicated on the label. The reagents must not be used after the expiry date. It is advisable to minimize their time left outside the refrigerator and to avoid leaving them at room temperature between uses.

REAGENTS AND MATERIALS NECESSARY

- Plastic and glass test tubes, tube rack.
- Automatic pipettes with adjustable precision.
- Centrifuge of relative force from 100 to 1200 g.
- Isotonic saline solution (0.9 % NaCl).
- PALERM (DIAGAST ref.: see catalogue).
- Positive control blood samples of guaranteed phenotype are required carrying the corresponding heterozygous antigen (except for the Lewis system) and similarly for a negative control, blood samples should be used which lack the antigen corresponding to the reagent used.
- Negative control: NEG CONTROL (DIAGAST ref.: see catalogue).

SAMPLES – CONTROLS

TEST BLOOD SAMPLE

The blood sample, collected in E.D.T.A., heparin or citrate anticoagulant, in a stoppered sterile tube and stored between +2ºC and +8ºC, should be tested within 72 hours, providing that no haemolysis is visible.
At the time of testing, centrifuge the blood sample at 1200 g for 3 minutes.

BLOOD SAMPLES OF GUARANTEED PHENOTYPE

The use of these control samples ensures the detection of anomalies with (handling, reagents, apparatus and the environment) and enables the user to implement any corrective actions.
The analytical system must be validated using control samples of guaranteed phenotype :

- sample expressing the antigen corresponding to the reagent antibody used,
- sample lacking the antigen corresponding to the reagent antibody used.

For each blood sample to be tested, a reagent control is performed alongside using the same conditions but replacing the reagent with NEG CONTROL.

PROCEDURE FOR ANTI-Cw (RH8), ANTI-Fyb (FY2), ANTI-Jka (JK1), ANTI-Jkb (JK2), ANTI-M (MNS1) AND ANTI-N (MNS2)

- In a plastic tube, prepare a 5 % unwashed red blood cell suspension in isotonic saline solution.
- Using the vial dropper, transfer 1 drop of reagent to a glass tube.
- Add 50 µL of erythrocyte suspension.
- Shake to mix, then centrifuge at 500 g for 1 minute.
- Gently swirl the tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
- Read and record the reaction immediately.

PROCEDURE FOR ANTI-Lea (LE1)

- In a plastic tube, prepare a 5 % unwashed red blood cell suspension in isotonic saline solution.
- Using the vial dropper, transfer 1 drop of reagent to a glass tube.
- Add 50 µL of erythrocyte suspension.
- Using the vial dropper, transfer 1 drop of PALERM.
- Incubate the tubes between +18ºC and +25ºC for 5 minutes.
- Shake to mix, then centrifuge at 500 g for 1 minute.
- Gently swirl the tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
- Read and record the reaction immediately.

PROCEDURE FOR ANTI-Leb (LE2)

- In a plastic tube, prepare a 5 % unwashed red blood cell suspension in isotonic saline solution.
- Using the vial dropper, transfer 1 drop of reagent to a glass tube.
- Add 50 µL of erythrocyte suspension.
- Incubate the tubes between +18ºC and +25ºC for 15 minutes.
- Shake to mix, then centrifuge at 500 g for 1 minute.
- Gently swirl the tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
- Read and record the reaction immediately.

PROCEDURE FOR ANTI-P1

- In a plastic tube, prepare a 5 % unwashed red blood cell suspension in isotonic saline solution.
- Using the vial dropper, transfer 1 drop of reagent to a glass tube.
- Add 50 µL of erythrocyte suspension.
- Shake to mix, then centrifuge at 120 g for 1 minute.
- Gently swirl the tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
- Read and record the reaction immediately.
- If no agglutination is visible, incubate the tubes between +2ºC and +8ºC for 30 minutes.
- Shake to mix, then centrifuge at 120 g for 1 minute.
- Gently swirl the tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
- Read and record the reaction immediately.

INTERPRETATION

If agglutination is present (the red blood cells form one or several clump(s)), the reaction is positive and the antigen corresponding to the reagent used is present on the test red blood cells.
If there is no agglutination (the red blood cells go back into homogeneous suspension), the reaction is negative and the antigen is absent from the test red blood cells.
The reaction can only be interpreted if :

- the result of the NEG CONTROL with the subject's red blood cells is negative,
- the analytical system has been validated with samples of guaranteed phenotype.

LIMITATIONS OF THE METHOD

Only suitably qualified personnel should use the devices.
It is advisable to gently shake the reaction tubes with ANTI-Jka (JK1), because the agglutinations created by ANTI-Jka (JK1) are more brittle.
Temperatures > 25 ºC can disrupt results obtained with ANTI-M (MNS1) and/or ANTI-N (MNS2). It is therefore advisable to take these reagents out of the refrigerator just before use and to read the reactions immediately after centrifugation.
It is imperative to use the calibrated dropper of the IVDMD vial to transfer 1 drop of reagent.
Only use the complementary reagents cited in the section entitled 'Reagents and materials necessary'.
The reactions are to be read immediately after centrifuging and resuspending.
It is imperative to work with clean apparatus and uncontaminated products (bacterial or other contamination).
Strict compliance with the following is required:

- storage conditions and expiry date,
- the procedure indicated above,
- equipment calibration is recommended.

No reagent can guarantee the detection of all the antigenic profiles rare, weak or variants.

PERFORMANCE DATA

A performance assessment of the reagents was conducted on a random samples panel of known common phenotypes including clinical and neonatal samples. The samples were drawn in the recommended anticoagulants (E.D.T.A., heparin, citrate). The expert assessment demonstrated 100 % specificity for each of the reagents with respect to the expected results.
ANTI-M (MNS1) can recognize unspecifically some HENSCHAW red blood cells (M –, He + is an extremely rare phenotype).
On 25 blood samples from blood donors having a FYX phenotype, the ANTI-Fyb (FY2) PK has detected 100% of these red blood cells.
Performances of devices are guaranteed for the method when used in combination with reagents recommended in this leaflet (for instance : PALERM).
Use and validation of other reagents used in combination with the devices, other than those indicated in paragraph titled 'Reagents and materials necessary' is possible but only on the user' s responsibility.

DIAGAST denies all responsibility in cases where the devices are not used in accordance with this leaflet.

REVISION HISTORY

Description of the change	Impact on the Verification of method according to standard NF EN ISO 15189
§ « Composition » : - Replacement IgM by IgG for ANTI-M and ANTI-N	No

CE 0459

The identifier, '0459', of the notified organization (LNE/G-MED) which validated the conformity evaluation procedure, cited in appendix IV of Directive 98/79/EC, is only applicable to the ANTI-Jka (JK1), ANTI-Jkb (JK2) and ANTI-Fyb (FY2) antibodies. The ANTI-Jka (JK1), ANTI-Jkb (JK2) and ANTI-Fyb (FY2) antibodies are included in list B of appendix II to that directive.

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