BLOOD GROUPING REAGENTS

Anti-Fy^b (FY2) — **REF 210532**

Anti-Jk^a (JK1) / Anti-Jk^b (JK2) — **REF 210534 / REF 210535**

(Human/Murine Monoclonal)

Anti-M (MNS1) — **REF 210536**

ANTI-P_1 — **REF 210540**

Anti-Le^a (LE1) / Anti-Le^b (LE2) — **REF 210541 / REF 210542**

(Murine Monoclonal)

For tube technique

- For *In Vitro Diagnostic Use*
- Meets FDA potency requirements
- Discard if turbid
- Preservative:<0.1% (w/v) sodium azide

INTENDED USE

These reagents are designed to determine the presence of blood group antigens, Fy^b (FY2), Jk^a (JK1), Jk^b (JK2), Le^a (LE1), Le^b (LE2), M (MNS1), and P_1 (P1), on the surface of human red blood cells by manual method.

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION

The Duffy-b (Fy^b) antigen was named in 1951 when found to be antithetical to Fy^a, first identified in 1950, from antibody in the serum of a multi-transfused hemophilic patient.
Duffy antibodies are implicated in mild to severe HTR or mild HDN.
The Fy^b antigen is a poor immunogen comparatively to Fy^a antigen.

The Kidd-a (Jk^a) antigen was first identified in 1951 when the corresponding antibody was found to cause hemolytic disease of the fetus and newborn (HDN). The Jk^b antigen was found in 1953 and named as antithetical antigen to Jk^a.
Kidd antibodies have been shown to be implicated in severe delayed hemolytic transfusion reactions (HTR).

The Lewis antigens are carbohydrates on glycolipids and are not intrinsic to the red blood cells but are absorbed onto membrane from plasma circulating lipoproteins.
Lewis antibodies are rarely implicated in HTR or HDN.

The M antigen was identified in 1927 as the first antigen of the MNS system, and is carried on glycophorin molecules.
M antibodies, mostly of the IgM class are rarely implicated on HTR and HDN when of the IgG class.

The P1 antigen was discovered in 1927, and is located on glycolipids.
P1 antibodies, mostly of the IgM class does not cause HDN but has rarely implicated on HTR.

PRINCIPLE OF THE TEST

The manual technique employed in a tube, utilizes the principle of hemagglutination. Test red blood cells bearing an antigen agglutinate in the presence of the reagent containing the corresponding antibody:

- either using the direct hemagglutination method,
- or using papain, proteolytic enzyme derived from the papaya (Carica papaya) that 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.

REAGENTS

All the reagents contain sodium azide (<0.1%). All the reagents except Anti-P_1 and Anti-Fyb (FY2) contain sodium arsenite (0.02%). Anti-Fyb (FY2) contains Proclin at 0.055%. All reagents contain bovine materials. Any bovine materials used in the manufacture of these products are sourced from donor animals that have been inspected and certified by Veterinary Service inspectors to be disease free.

These reagents are produced by DIAGAST from monoclonal antibodies derived from the *in vitro* culture supernatant of human/murine heterohybridomas (Anti- Fyb, Anti-Jka and Anti-Jkb) or murine hybridomas (Anti-Lea, Anti-Leb, Anti-M and Anti-P1).

The reagents are provided with calibrated droppers.

Code	Product Designation	Packaging
210532	Anti-Fyb (FY2)	1 x 3 ml
210534	Anti-Jka (JK1)	1 x 3 ml
210535	Anti-Jkb (JK2)	1 x 3 ml
210536	Anti-M (MNS1)	1 x 3 ml
210540	Anti-P_1	1 x 3 ml
210541	Anti-Lea (LE1)	1 x 3 ml
210542	Anti-Leb (LE2)	1 x 3 ml

WARNINGS AND PRECAUTIONS

- These reagents contain <0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide build-up. Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.
- 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.
- For *In Vitro Diagnostic Use.*
- Do not use beyond expiration date.
- Do not use damaged or leaking reagents.
- Do not use if turbid.
- Do not dilute.
- The absence of all viruses has not been determined in these reagents.
- These reagents have components (Dropper bulb) containing dry natural rubber which may cause allergic reactions.
- These reagents contain material of human or animal origin and may transmit infectious agents and should be handled with extreme caution. No known test method can offer complete assurance that products derived from human sources will not transmit infectious agents.
- CAUTION for Anti-Fyb: SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS. APPROPRIATE CARE SHOULD BE TAKEN IN THE USE AND DISPOSAL OF THIS PRODUCT.

WARNING 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.

STORAGE AND STABILITY

- Store reagents at 2°C to 8°C when not use. Do not freeze.
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to the specimen collection.
The blood samples collected following standard blood sampling guidelines in EDTA, heparin or sodium citrate anticoagulant should be stored at 2-8°C.
They should be tested within the following validated hold times:
- Clotted specimens or blood drawn into sodium citrate or EDTA should be tested within 7 days.
- Blood drawn into heparin should be tested within 2 days.

Red blood cells from bags collected in ACD, ACD with AS-1, CPD, CPD with AS-1, CPDA-1, CP2D and CP2D with AS-3 can also be used up to 7 days after the expiration date indicated on the label of the bag.
Do not use blood specimens that exhibit contamination.

MATERIALS:

Material provided:
- Anti-Fyb (FY2) (REF 210532): Monoclonal antibody. Anti-Fyb IgM human/murine clone SpA264LBg1.
- Anti-Jka (JK1) (REF 210534): Monoclonal antibody. Anti-Jka IgM human/murine clone P3HT7.
- Anti-Jkb (JK2) (REF 210535): Monoclonal antibody. Anti-Jkb IgM human/murine clone P3.143.
- Anti-M (MNS1) (REF 210536): Monoclonal antibody. Anti-M IgG murine clone 2514E6.
- Anti-P1 (P1) (REF 210540): Monoclonal antibody. Anti-P1 IgM murine clone 650.
- Anti-Lea (LE1) (REF 210541): Monoclonal antibody. Anti-Lea IgM murine clone 13643B9.
- Anti-Leb (LE2) (REF 210542): Monoclonal antibody. Anti-Leb IgM murine clone GX336.

Material required but not provided:
- Test tubes, tube rack.
- Pasteur pipettes (drop volume 40 to 50 µl) or Automatic pipettes with adjustable precision.
- Centrifuge of relative force from 100 to 1200 rcf.
- Timer
- Isotonic saline solution (0.9% NaCl).
- 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.

Other required complementary reagents:
- Papain (REF 210545).

TEST PROCEDURES

- **For Anti-Fy^{b} (FY2), Anti-Jk^{a} (JK1), Anti-Jk^{b} (JK2) and Anti-M (MNS1)**

1. In a test tube, prepare a 3-5% unwashed red blood cell suspension in isotonic saline solution.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.
4. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration centrifuge.
5. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
6. Read and record the reaction immediately. It is recommended grading positive reactions.

- **For Anti-P$_1$ (P1)**

1. In a test tube, prepare a 3-5% unwashed red blood cell suspension in isotonic saline solution.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.

4. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration centrifuge.
5. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
6. Read and record the reaction immediately. It is recommended grading positive reactions.
7. If no agglutination is visible, incubate the test tubes at 2-8ºC for 30 minutes.
8. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration centrifuge.
9. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
10. Read and record the reaction immediately.

- **For Anti-Le^{a} (LE1)**

1. In a test tube, prepare a 3-5% unwashed red blood cell suspension in isotonic saline solution.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.
4. Using the vial dropper, transfer 1 drop of Papain.
5. Incubate the test tubes at 18-25ºC for 5 minutes.
6. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration centrifuge.
7. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
8. Read and record the reaction immediately. It is recommended grading positive reactions.

- **For Anti-Le^{b} (LE2)**

1. In a test tube, prepare a 3-5% unwashed red blood cell suspension in isotonic saline solution.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.
4. Incubate the test tubes at 18-25ºC for 15 minutes.
5. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration centrifuge.
6. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
7. Read and record the reaction immediately. It is recommended grading positive reactions.

RESULTS

Positive result: 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.

Negative result: If there is no agglutination (the red blood cells reform a homogeneous suspension), the reaction is negative and the antigen is not present on the tested red blood cells.
For Anti-M (MNS1), after centrifugation while resuspending RBC, some negative samples could show fragile aggregates: continue to resuspend until all RBC are dispersed. Agglutination of positive samples will not be affected.

Interpretation:
The reaction can only be interpreted if the analytical system has been validated with control samples of guaranteed extended phenotype.

QUALITY CONTROLS

The use of samples of guaranteed extended phenotyping as control samples allows the user to detect anomalies with (handling, reagents, apparatus and the environment) and to implement corrective actions as required.
Known samples control should be run in parallel on each day of use.
- a sample possessing the antigen corresponding to the antibody in the reagent used,
- a sample devoid of the antigen corresponding to the antibody in the reagent used.

If an unexpected control result is obtained, a complete assessment of the reagents and material used should be made.

LIMITATIONS OF THE PROCEDURE

- These reagents are not to be used in a method not described in this Instructions For Use.

- Only use the complementary reagents cited in the section entitled “MATERIAL - Other required complementary reagents “.
- 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 >25ºC can disrupt results obtained with Anti-M (MNS1). 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 recommended to use the calibrated dropper provided in the vial to dispense a reagent drop.
- The reactions are to be read immediately after centrifuging and resuspending.
- False positive or false negative can occur due to improper centrifugation.
- It is imperative to work with clean apparatus and uncontaminated products (bacterial or other contamination).
- Strict compliance with the following is required:
  - storage conditions,
  - equipment calibration is recommended.
- No reagent can guarantee the detection of all the antigenic profiles rare, weak or variants.

SPECIFIC PERFORMANCE CHARACTERISTICS

- These reagents meet FDA potency requirements for Blood Grouping Reagents to be used in tube technique.
- Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
- The device performance is guaranteed only if they are used in the proposed technique and reagents to be used in combination mentioned in this Instructions For Use (ex : Papain REF 210545)
- Use and validation of other reagents used in combination with the devices, other than those indicated in paragraph titled " MATERIAL - Other required complementary reagents”, 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 Instructions For Use.
- Anti-M can recognize unspecifically some HENSCHAW red blood cells (M-, He+ is an extremely rare phenotype).
  - The performance of the reagents was confirmed against FDA-licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites. The estimated percent agreements and their lower limits of 95% one-side confidence interval for all sites combined are indicated on the table below.

Table 1. Overall Statistical Analysis results of the comparison study

		N	Lower 95% CI	Percent Agreement	Acceptance Criteria
Anti-Fy[b]	NPA	345	98.63%	99.71%	99%
	PPA	930	99.17%	99.68%	99%
Anti-Jk[a]	NPA	264	98.22%	99.62%	99%
	PPA	1007	99.53%	99.90%	99%
Anti-Jk[b]	NPA	387	98.78%	99.74%	99%
	PPA	884	99.13%	99.66%	99%
Anti-M	NPA	244	98.78%	100%	99%
	PPA	1028	99.71%	100%	99%
Anti-P1	NPA	271	96.16%	98.15%	99%
	PPA	1000	99.70%	100%	99%
Anti-Le[a]	NPA	1009	99.70%	100%	99%
	PPA	260	98.19%	99.62%	99%
Anti-Le[b]	NPA	403	98.83%	99.75%	99%
	PPA	867	99.45%	99.88%	99%

Percent of Agreement only indicates agreement between the DIAGAST reagents and the FDA-licensed reagents and does not indicate which reagent gave the correct result(s).

BIBLIOGRAPHY

- Technical Manual. 18th ed. Bethesda, MD: American Association of Blood Banks, 2014.
- Standards for Blood Banks and Transfusion Services. 30th ed. Bethesda, MD: American Association of Blood Banks, 2016.

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SYMBOLS KEY

One of more of these symbols may have been used in the labeling/packaging of this product.