

## **Red Cell EZ TYPE<sup>®</sup>** **(Rare Screen, Rare ID)**

### **INTENDED USE**

Red Cell EZ TYPE<sup>®</sup> is an assay for the molecular determination of alleles of blood group antigen systems, using PCR amplification of human genomic DNA.

- **Rare ID** types for alleles of the Kp, Lutheran (Lu), Diego (Di), Wright (Wr), Cartwright (Yt), Colton (Co), Knops (Kn) and Dombrock (Do) blood group systems.
- **Rare Screen** detects one or more of Kp<sup>a</sup> (KEL3), Lu<sup>a</sup> (LU1), Yt<sup>b</sup> (Y2), Co<sup>b</sup> (CO2), or Kn<sup>b</sup> (KN2) in a single PCR reaction.

*For Research Use Only*

### **SUMMARY AND EXPLANATION OF THE TEST**

The established method for determination of blood group antigens is serologic typing. Recently the genetic organization of many genes that code for blood group antigens has been elucidated, and the genetic basis for many blood group antigens determined. This knowledge has made possible DNA-based typing for the polymorphisms that encode blood group antigens. DNA-based typing cannot entirely replace serologic methods. The genetic basis for at least some of the antigens in these systems remain unidentified, and unknown genetic variation may cause a predicted antigen to be absent in fact. However, DNA-based typing can resolve questions that have proven to be difficult for serological methods to answer due to lack of appropriate cell panels or typing sera. Further, DNA-based typing can readily assess zygosity and can illuminate issues of antigen expression, both of which are difficult to resolve with serological methods.

DNA-based typing of polymorphisms for blood group antigens following PCR amplification can be performed by a variety of methods: RFLP assays, SSO probe assays, or allele-specific amplification with gel detection (SSP). Of these, PCR-SSP analysis, the basis of the Red Cell EZ TYPE<sup>®</sup> assays, offers improved assay speed and lower hands-on time. The PCR-SSP method only requires two working steps (amplification and detection) because both amplification and discrimination occur during the PCR process.<sup>6</sup> Amplification of the polymorphic sequences of interest is followed by detection with agarose gel electrophoresis. This inherent assay utility is further enhanced by the inclusion of precast agarose gels in the test system. These cassette-enclosed gels eliminate the need for gel casting, buffer preparation, and minimize issues related to mutagenic DNA stains (ethidium bromide) in gels or electrophoresis buffer.

### **PRINCIPLE OF THE PROCEDURE**

Red Cell EZ TYPE<sup>®</sup> assays are based on the *Polymerase Chain Reaction* (PCR), which enables amplification of defined target sequences in the genomic DNA.<sup>2</sup> After successful amplification the sample contains the target DNA sequence in quantities sufficient for detection. Sequence-Specific Priming (SSP) describes a specific type of PCR in which amplification occurs only if the allele is present; samples lacking the target for an allele-specific primer set do not produce that particular PCR product.<sup>3,4</sup> To be useful, PCR-SSP analysis requires that a number of amplifications be carried out in parallel. Amplification of internal control primers targeted to the Human Growth Hormone (HGH) gene demonstrates acceptable reaction conditions for each PCR tube. If no allele-specific product is present after PCR, the product of this internal control must be clearly detectable. Negative Control tubes detect exogenous DNA contamination if present. PCR products are separated by agarose gel electrophoresis on pre-cast E-Gels<sup>®</sup>, during which the PCR products are also stained by ethidium bromide. The separated products are then viewed on an ultraviolet (UV) transilluminator. A gel image may be captured by film or digital photography as a record of the assay. Determination of alleles is performed by comparing the pattern of allele-specific bands from the gel image to the patterns identified on the corresponding Recording Sheet.

### **SCREEN, ID FOR RARE ANTIGENS**

The Red Cell EZ TYPE<sup>®</sup> Rare Screen and Rare ID assays enable screening and subsequent identification of the alleles of the Kp, Lutheran (Lu), Diego (Di), Wright (Wr), Cartwright (Yt), Colton (Co), Knops (Kn) and Dombrock (Do) blood group systems. These blood group systems are biallelic with one frequent and one rare allele. The Dombrock system is an exception in that both alleles occur with nearly equal frequency.

In the Red Cell EZ TYPE<sup>®</sup> **Rare Screen**, one PCR reaction per sample is performed; a positive result indicates the presence of the rare allele for at least one of five antigens: Kp<sup>a</sup>, Lu<sup>a</sup>, Yt<sup>b</sup>, Co<sup>b</sup>, Kn<sup>b</sup>. The rare allele can then be identified using the **Red Cell EZ TYPE<sup>®</sup> Rare ID** assay. This PCR SSP assay detects homozygosity or heterozygosity for all 8 systems: KEL3/KEL4 (Kp<sup>a</sup>/Kp<sup>b</sup>); Lutheran (Lu<sup>a</sup>/Lu<sup>b</sup>); Diego (Di<sup>a</sup>/Di<sup>b</sup>); Wright (Wr<sup>a</sup>/Wr<sup>b</sup>); Colton (Co<sup>a</sup>/Co<sup>b</sup>); Cartwright (Yt<sup>a</sup>/Yt<sup>b</sup>); Knops (Kn<sup>a</sup>/Kn<sup>b</sup>); Dombrock (Do<sup>a</sup>/Do<sup>b</sup>) using 16 different PCR reactions. Table 1 below gives an overview of the blood group systems contained in this kit. The details are derived from "Human Blood Groups" (2002) by Geoff Daniels and refer to the named population groups. The allele frequencies of the rare allele variants are outlined in grey.

**Table 1 Rare Antigens Systems**

NAME	SHORT	Chromosome	Serol.	DNA	Allele FRQ.	Population group	Serol.	DNA	Allele FRQ.
Kell	Kp	7q33	<b>Kp<sup>a</sup></b>	<b>KEL3</b>	0.0114	Europe	<b>Kp<sup>b</sup></b>	<b>KEL4</b>	0.9886
Lutheran	Lu	19q13.2	<b>Lu<sup>a</sup></b>	<b>LU1</b>	0.0390	Europe	<b>Lu<sup>b</sup></b>	<b>LU2</b>	0.9610
Diego	Di	17q12-q21	<b>Di<sup>a</sup></b>	<b>DI1</b>	0.0531	Japan	<b>Di<sup>b</sup></b>	<b>DI2</b>	0.9469
Wright	Wr	17q12-q21	<b>Wr<sup>a</sup></b>	<b>DI3</b>	0.0003	Europe (Switzerland)	<b>Wr<sup>b</sup></b>	<b>DI4</b>	0.9997
Colton	Co	7p14	<b>Co<sup>a</sup></b>	<b>CO1</b>	0.9561	Europe (Commonwealth)	<b>Co<sup>b</sup></b>	<b>CO2</b>	0.0439
Cartwright	Yt	7q22.1	<b>Yt<sup>a</sup></b>	<b>Y1</b>	0.9587	Europe	<b>Yt<sup>b</sup></b>	<b>Y2</b>	0.0413
Knops	Kn	1q32.1-3	<b>Kn<sup>a</sup></b>	<b>KN1</b>	0.9900	Europe	<b>Kn<sup>b</sup></b>	<b>KN2</b>	0.0100
Dombrock	Do	12p13.2-12.1	<b>Do<sup>a</sup></b>	<b>DO1</b>	0.4200	Europe	<b>Do<sup>b</sup></b>	<b>DO2</b>	0.5800

**REAGENTS**

Maximum number of tests per kit:

- 12 typings per kit (Rare ID), 96 tests per kit (Rare Screen)

- |            |  |
|------------|--|
| <b>PP</b>  | <b>Primer Plate (Rare ID):</b> 96-tube PCR trays with allele-specific primers for the antigen systems described in Table 1, and Internal control (HGH) primers, dried on the inner surface of tubes. Sealed with foil covers. Ready for use.   |
| <b>PT</b>  | <b>Primer Tubes (Rare Screen):</b> 1 x 8 PCR tube strips (12) with allele-specific (Kp <sup>a</sup> , Lu <sup>a</sup> , Yt <sup>b</sup> , Co <sup>b</sup> , Kn <sup>b</sup> ) primers and internal control (HGH) primers dried on the inner surface of tubes. Each tube is sealed by an individual cap. Ready for use. |
| <b>PR</b>  | <b>PCR Reagent:</b> A buffer containing magnesium chloride, free deoxynucleotide triphosphates (dNTPs), glycerol, cresol red, and other proprietary components. Dilute before use.   |
| <b>NCT</b> | <b>Negative Control Tubes:</b> PCR tubes containing dried primers, sealed with tube caps. Ready for use.   |
- E-Gel®** **E-Gel®:** precast agarose gels (2% 16 well, 2% 48 well, or 2% 96 well) used for separation of the PCR products. Ready for use. Supplied separately.

**PRECAUTIONS**

- Do not use reagents or gels beyond their expiration date.
- Do not use reagents that are turbid or contaminated.
- Do not use PCR Reagent that has changed color from red to yellow.
- Do not use gels that appear cracked, dried out, or show evidence of having been frozen.
- PCR tubes and reagents contained in the kit should not be used in conjunction with any other test system.
- Due to variations in the performance of different thermal cyclers, it may be necessary for the laboratory to establish adjusted parameters for the thermal cycling program in order to achieve valid results. It may also be necessary to determine the appropriate spacer pad to insure complete closure of the foil sealer during PCR.
- Do not isolate DNA samples from heparinized blood. Heparin may interfere with the PCR amplification.
- **PCR Laboratory Practice**  
 PCR is an extremely powerful method of amplifying even the smallest amounts of DNA. Extraordinary precautions must be adhered to in order to avoid contamination with spurious genomic DNA or PCR product. Of particular importance is the avoidance of contamination by PCR product from previous amplifications. The following precautions are of special importance:
  - Spatial separation of the pre-PCR area (DNA isolation and storage, PCR setup) from the post-PCR area (thermal cycler, gel loading and electrophoresis, evaluation). Instruments and consumables from post-PCR areas must not be taken into the pre-PCR area.
  - Use of pipettes with aerosol protection (sterile barrier tips) in both the pre and post-PCR areas.
  - The Negative Control reaction is an environmental control designed to detect DNA contamination and should always be run in parallel with sample amplifications.

**CAUTION**

- When finished with the assay, dispose of waste materials as biohazardous waste and decontaminate non-disposable materials with 10% household bleach or other DNA inactivating agent.
- The UV transilluminators used to visualize the PCR product bands emit powerful ultraviolet light which can damage eyes and skin. Always wear protective clothing and UV-blocking glasses or face shield when operating the UV transilluminator.

- E-Gel<sup>®</sup>s contain a small amount of ethidium bromide as a DNA stain. Ethidium bromide is a known human mutagen. Do not open the gel cassettes. Dispose of gels according to local hazardous waste regulations.

## **SPECIMEN COLLECTION**

- Isolate DNA using a published method or a commercial kit that can deliver DNA samples with a 260 nm/280 nm ratio of 1.60 – 2.0 and concentration in the range of 25 – 100 ng/μL.
- Resuspend the DNA in sterile water or 10 mM Tris, pH 8.0 -9.0. Samples should **not** be rehydrated in solutions containing greater than 0.5 mM EDTA or other chelating agents. These may interfere with PCR.
- DNA samples may be assayed immediately after isolation or stored in a non-defrosting freezer at or below -20°C for an extended period (up to 5 years) without affecting results.
- Presence of excess contaminating protein, RNA, heparin, EDTA, or other chelating agents may interfere with PCR amplification of the purified DNA.

## **PROCEDURE**

### **Materials Provided**

#### *PCR Components (≤ -20°C storage)*

- 2 x 96-tube clear PCR trays sealed with labelled foil covers (Rare ID) or 96 PCR tubes (12 1x8 strips) enclosed in a labelled bag (Rare Screen). Primer Tubes contain allele-specific and the internal control primers. The Rare ID plates are cut so that “blocks” of PCR tubes for each assay (2 strips/assay) can be readily separated. Tubes for Rare Screen are sealed by individual caps.
- 1 x 500 μL (Rare Screen) or 2 x 500 μL (Rare ID) PCR Reagent.
- Twelve (12) Negative Control PCR tubes in a labelled resealable bag.
- 2 resealable bags, each containing 12 1x8 cap strips (Rare ID). Rare Screen Primer Tubes have attached individual caps.

**RSC**

#### *Gels (15 to 30°C storage)*

- E-Gel 16 (6 x 2% double-comb E-Gel<sup>®</sup> per box)  
OR  
E-Gel 48 (8 x 2% E-Gel<sup>®</sup> 48 per box)  
OR  
E-Gel 96 (3 x 2% E-Gel<sup>®</sup> 96 per box)

Users who prefer heat sealing of the PCR tubes require the following 2 additional items, which can be obtained from GTI.

Thermo sealing foils  
1mm silicone spacer pad

### **Materials Required (but not provided)**

#### A. For PCR

- Taq Polymerase, either native or recombinant, 5U/μL. Do not use other thermostable polymerases or “hot-start” preparations of Taq polymerase.

The following enzymes have been validated for use with Red Cell EZ TYPE<sup>®</sup>. Use of other enzymes must be validated by the user.

**Table 1**

<b>Supplier</b>	<b>Product</b>
Promega	GoTaq Flexi (M8296)
Applied Biosystems	AmpliTaq (Product #N8080172)
Inno-Train	AxiTaq

- Programmable thermal cycler with block sized to accept 96 x 0.2 mL PCR tubes.
- Adjustable micropipets to deliver 1 - 1000 μL and sterile barrier tips
- Molecular Biology grade water (DNA- and DNase-free)
- Ice bath or cold blocks to fit 0.6 mL or 1.5 mL tubes
- PCR (0.2 mL) tube rack
- Vortex mixer
- Snap-top conical polypropylene microcentrifuge tubes (0.5 - 1.7 mL), DNA- and DNase free
- 10% bleach or other DNA-inactivating agent

#### *Optional*

- Electronic pipettor capable of delivering 10 μL
- Cap roller/setter (if using cap strips to seal PCR tubes)
- Heat sealing device (for use with thermo sealing foils)
- Sealing roller (if using thermo sealing foil to seal PCR tubes)

*B. For Electrophoresis and Analysis*

- E-Gel<sup>®</sup> iBase<sup>™</sup> or Mother E-Base<sup>™</sup>
- PCR (0.2 mL) tube rack
- Adjustable micropipets to deliver 1 - 20 µL, and sterile barrier tips
- Plastic-backed absorbent paper for lab bench
- UV transilluminator (GTI cat. #EZT-ILLUM)
- 10% bleach or other DNA inactivating agent
- Deionized water
- Gel documentation system (GTI cat.# EZT-CAMHOOD)

*Optional*

- E-Ladder (GTI Cat. # ELAD)
- 8-channel multichannel pipettor capable of delivering 5-20 µL

**Test Procedure**

*A. In Advance*

1. Isolate genomic DNA from all samples to be tested. Each sample should have a DNA concentration in the range of 25-100 ng/µL and a 260 nm/280 nm ratio of 1.60 – 2.0.
2. Program a thermal cycler with the **Red Cell EZ TYPE<sup>®</sup> Rare Screen/ID PCR** program, which is used only for the Rare Screen and Rare ID assays. The program is as follows:

**Table 2 Thermal Cycling Program**

<b>Initial</b>	<b>10 cycles</b>	<b>20 cycles</b>	<b>72°C Hold</b>	<b>End</b>
94° 2 minutes	94° 20 seconds 65° 60 seconds	94° 20 seconds 61° 60 seconds 72° 30 seconds	72° 5 minutes	10° Hold

**NOTE:** *This cycling program has been tested with thermal cyclers from Applied Biosystems (PE9700, PE9600) and Bio-Rad (PTC-100, PTC-200). For the PE9700 the ramping rate should be limited to ~1°C/second (9600 mode); however, the PTC-200 need not be rate controlled for ramping. Amplification on other thermal cyclers may require optimization of the cycling program.*

*B. PCR*

3. Turn on the thermal cycler in advance of setup to ensure that the heated lid has time to reach its operating temperature. Check the cycling program to be sure it has not been changed.
4. Prepare work surfaces and pipettors before use by wiping with 10% household bleach or other DNA-inactivating agent.
5. If heat sealing will be used, turn on the heat seal device and allow it to come to operating temperature (5 – 7 min).
6. Determine the numbers of samples to be tested. Remove the required number of Primer Plate blocks (Rare ID) or Primer Tubes (Rare Screen), Taq polymerase, Negative Control tubes, and sufficient PCR Reagent from the freezer. Thaw PCR Reagent at room temperature. Keep the Taq polymerase and PCR Reagent on ice or in a cold block. Place the Primer Plate blocks (Rare ID) or Primer Tubes (Rare Screen) and the Negative Control tube(s) in a tube rack.

**NOTE:** *If more than one DNA sample is being tested with a particular assay, only one Negative Control reaction is required. Similarly, if more than one assay is being run on a DNA sample only one Negative Control reaction is required.*

7. Fill out the Red Cell EZ TYPE<sup>®</sup> Rare Screen/Rare ID PCR Setup Protocol, using the appropriate side. The Protocol will indicate the required volumes of reagents for the number of assays being performed.
8. Prepare the master mix or master mixes as calculated on the Red Cell EZ TYPE<sup>®</sup> Rare Screen/Rare ID PCR Setup Protocol. Keep master mixes on ice or in a cold block.
9. Record the kit lot number and expiration date on a copy of the kit Recording Sheet. For Rare ID, remove the foil sealer from the primer plate blocks and discard. Label each block of tubes with an identifier for one of the samples to be tested. Primer mix 1 is in the tube with the black mark. Pipet 10 µL of the appropriate DNA-containing master mix into each tube of a block of Primer Tubes.

**NOTE:** *It is critical to maintain awareness of the orientation of each Primer Tube “block”, so that the tube order is known throughout the assay process. For each tube “block” tube 1 is marked by a black dot. Similarly, awareness of the orientation of the primer tube strips for Rare Screen must be maintained so that assay results can be accurately assigned.*

10. Seal the Rare ID tubes by pressing cap strips into place, and go to step 12. Heat sealing of the PCR tubes is described in step 11.
11. Heat sealing can only be used with the Rare ID strips. Place the blocks of primer tubes and the negative control tube into the bottom rack of the heat sealer. Cut a section of thermo-sealing foil large enough to cover the primer tube blocks including the negative control tubes (maximum 5 samples for Rare ID), and center the section on top of the primer tubes. Lower the heating element to the plate and press, holding for 3 to 5 seconds. Release the heating element and run a finger over the tops of the tubes to aid in sealing. A rubber sealing roller may also be used to seat the foil. Remove the plate from the sealer.

**NOTE:** *Sealing for longer than 5 seconds or excessive pressure during the sealing operation can narrow the opening of the PCR tubes, making retrieval of the amplified products difficult.*

12. Examine the PCR tubes after sealing. Dislodge any bubbles in the bottoms of PCR tubes, and force any hanging drops down into the reaction volume. This may be performed by gently tapping tubes on the benchtop or by a brief centrifugation in a swinging-bucket centrifuge equipped with a microwell plate carrier.
13. Start the Red Cell EZ TYPE<sup>®</sup> Rare Screen/ID program on the thermal cycler. Transfer the primer plate(s) from the rack to the block of the thermal cycler.
14. If the tubes were heat-sealed, place the supplied silicone spacer pad securely over the foil sealer to cover all of the PCR tubes. If less than a full block of tubes is being amplified, place empty PCR tubes at the unused corners and edges of the block as spacers. Slide the heated lid closed, making certain the silicone pad does not shift. Close the lid according to the thermal cycler manufacturer's instructions.

**NOTE:** *The silicone spacer pad is not required for tubes sealed with cap strips.*

15. When the thermal cycler has reached the last step of the program (the 10°C hold), transfer the primer plate to a PCR rack. Inspect each tube and note any that appear low in volume. Results of these tubes may be questionable. If electrophoresis will not be performed immediately, store the tubes refrigerated (2 to 8°C) for up to 3 days or in a freezer below -20°C for up to 7 days.

#### B. Detection

**NOTE:** *Detection steps must be performed in an area separate from that used as the pre-PCR area. Devices should not be shared between these areas to avoid carryover contamination.*

**NOTE:** *Detection may be carried out in either the E-Gel 16 (2% double-comb E-Gel<sup>®</sup>, 16 wells), E-Gel<sup>®</sup> 48 (2% double-comb gels, 48 wells), or E-Gel<sup>®</sup> 96 (2% gel, 96 wells) gels. Each E-Gel 16 gel can accommodate a single Rare ID assay or 16 Rare Screen assays. The E-Gel 48 or E-Gel 96 gels make possible the processing of multiple samples on larger gel formats.*

16. If the PCR tubes were stored frozen, remove them from the freezer and allow them to thaw prior to use.
17. Prepare the work area by setting down plastic-backed absorbent paper and setting up the iBase<sup>™</sup> or Mother E-Base<sup>™</sup>, deionized water, and pipettors at this spot. Move the PCR tubes and the required number of E-Gels to this work area.

**NOTE:** *Loading and running the E-Gel 16 gels is described in steps 18 – 26. Loading and running the E-Gel 48 gels is described in steps 27 – 35. Loading and running the E-Gel 96 gels is described in steps 36 – 45.*

#### **2% E-Gel 16 Loading and Running**

18. Open the pouch of a 2% double-comb E-Gel<sup>®</sup> and remove the gel. Insert the gel, right edge first, into the iBase. Press on the left edge at the top and bottom to seat the gel. Plug in the device.
19. Pre-run the gel. Press and hold the Mode button (M, second from right) until a blinking Program is observed. Use the Up and Down buttons to reach the Pre-run program (program 0). Press the Go button to begin the pre-run. The red LED that signals a properly-inserted gel turns to green when the run begins. The end of the pre-run is indicated by a flashing red light and a beep. Press the Go button to silence the alarm.
20. Label the gel's upper plate using a laboratory marker.
21. Remove the combs from the gel. Pipet 16 µL of deionized water to each well of the gel except the center half-wells.
22. Pipet the PCR products to the gel.
  - a. For Rare ID, open tube 1 (marked by a black dot) for the first sample, and pipet 6 µL of the contents into the assigned well of the gel. Repeat this process for each PCR tube and its corresponding well on the gel. Change pipet tips after each addition.

- b. For Rare Screen, open each tube and pipet 6  $\mu\text{L}$  of the contents into the assigned well of the gel. Change pipet tips after each addition.

**NOTE: Both processes are made easier by the use of an 8-channel multichannel pipettor capable of delivering 5-20  $\mu\text{L}$ .**

23. Pipet 5  $\mu\text{L}$  water to one of the center half-wells. Pipet 5  $\mu\text{L}$  of the negative control reaction to this well.
24. If a DNA ladder is desired as a size marker, pipet 10  $\mu\text{L}$  of E-Ladder to the other half well.

**NOTE: Other DNA size markers of the appropriate size range may be used. In this case the particular dilution and loading to the well must be determined by the user.**

25. Press the Mode (M) button and use the down arrow to select Program 3. The run time should be 15 minutes; if not, use the Up or Down arrow to adjust. Press the Go button to start electrophoresis. The red LED will change to green and the display will show the remaining time.
26. At the end of electrophoresis the green light will change to a red flashing light and an alarm will sound. Press the Power button to stop. The flashing red light will become a steady red light. Unplug the device and remove the gel from the iBase. Continue to step 46.

### **2% E-Gel<sup>®</sup> 48 Loading and Running**

27. Remove the E-Gel<sup>®</sup> 48 gel from its foil pouch and remove the combs. Clean the gel's upper plate with a damp laboratory wipe, if necessary. Label the gel's upper plate using a laboratory marker.
28. Pipet 12  $\mu\text{L}$  of deionized water to each well of the gel and the two M wells on the right side of the rows.
29. Pipet the PCR products to the gel.
  - a. For Rare ID, remove the first cap strip or open tube 1 (marked by a black dot), and pipet 6  $\mu\text{L}$  from the contents of tube 1 for the first sample into the assigned well of the gel. Repeat this process for each PCR tube and its corresponding well on the gel. Do the same for the other samples tested. Change pipet tips after each addition. Be sure that each sample's PCR tubes are pipetted to the assigned positions on the gel.
  - b. For Rare Screen, open each tube and pipet 6  $\mu\text{L}$  of the contents into the assigned well of the gel. Change pipet tips after each addition.
30. Pipet 6  $\mu\text{L}$  of the negative control product to one of the right-side M wells.
31. If a DNA ladder is desired as a size marker, pipet 15  $\mu\text{L}$  of E-Ladder to the two left side M wells.

**NOTE: Other DNA size markers of the appropriate size range may be used. In this case the particular dilution and loading to the well must be determined by the user.**

32. Plug in the Mother E-Base. A red light will come on and the time display will light up. Use the power button (right button) to toggle to EG mode (instead of EP mode).
33. Slide the gel under the two electrodes on the Mother E-Base and seat the gel so its electrodes make contact with the device electrodes.
34. Use the left button to set the timer for 15 minutes. Press the power button (right button) to begin electrophoresis. The red light will change to green while the run is in progress.
35. At the end of electrophoresis the green light will change to a red flashing light and an alarm will sound. Press the power button to stop the run. The flashing red light will become a steady red light. Unplug the device and remove the gel from the Motherbase. Continue to step 46.

### **2% E-Gel<sup>®</sup> 96 Loading and Running**

36. Remove the E-Gel<sup>®</sup> 96 gel from its foil pouch and remove the comb. Clean the gel's upper plate with a damp laboratory wipe, if necessary.
37. Label the gel's upper plate using a laboratory marker.
38. Pipet 16  $\mu\text{L}$  of deionized water to each well of the gel.
39. Pipet the PCR products to the gel.

- a. For Rare ID, open tube 1 (marked with a black dot) for the first sample and pipet 6  $\mu\text{L}$  into the assigned well of the gel. Repeat this process for each PCR tube for the first sample, and then for the remaining samples, with an aliquot of each tube's contents pipetted to its corresponding well on the gel. Change pipet tips after each addition. Be sure that each sample's PCR tubes are pipetted to the assigned positions on the gel.
- b. For Rare Screen, open each tube and pipet 6  $\mu\text{l}$  of the contents into the assigned well of the gel. Change pipet tips after each addition.

**NOTE:** *This process is greatly facilitated by the use of an 8-or 12-channel volume multichannel pipettor capable of delivering 5-20  $\mu\text{L}$ .*

40. Pipet 6  $\mu\text{l}$  of the negative control reaction to an unused well. If the plate is full, one of the right-side M wells may be used; in this case add 16  $\mu\text{l}$  deionized water to the well before adding the negative control volume.
41. If a DNA ladder is desired as a size marker, pipet 15  $\mu\text{l}$  of E-Ladder to each of the right-side M wells, except those used for negative controls.

**NOTE:** *Other DNA size markers of the appropriate size range may be used. In this case the particular dilution and loading to the well must be determined by the user.*

42. Plug in the Mother E-Base. A red light will come on and the time display will light up. Use the power button (right button) to toggle to EG mode (instead of EP mode).
43. Slide the gel under the two electrodes on the Mother E-Base and seat the gel so its electrodes make contact with the device electrodes.
44. Use the left button to set the timer for 11 minutes. Press the power button (right button) to begin electrophoresis. The red light will change to green while the run is in progress.
45. At the end of electrophoresis the green light will change to a red flashing light and an alarm will sound. Press the power button to stop the run. The flashing red light will become a steady red light. Unplug the device and remove the gel from the Motherbase.
46. Take the gel to the UV transilluminator for observation of product bands. The gel should be viewed and recorded within 20 minutes. If a record of the assay is desired, a photo of the gel should be taken using a gel documentation system.

### **QUALITY CONTROL**

A PCR product from the internal control primers (434 bp) should be detected in all amplifications except the negative control tubes. However, allele-specific amplification competes with amplification of the internal control, so that in amplifications positive for blood group-specific reactions the internal control product may be faint or absent. This is not cause for concern. However, if DNA was loaded to a PCR tube and no control **or** allele specific bands are observed, amplification failure is strongly indicated. Such amplification failures can result from deterioration of a reagent, an incorrectly-programmed thermal cycler, poorly purified sample DNA, insufficient quantity of sample DNA loaded to the PCR, a poorly sealed PCR tube, or failure to load a reactant to the PCR during setup. The negative control reaction should generate no detectable product bands. Presence of PCR products in this tube indicates some DNA contamination of the master mix. Such contamination may occur due to procedural errors or contamination of a reagent or tool used to set up the assay. If a negative control reaction is positive the assay results cannot be relied upon. The assay should be repeated.

### **INTERPRETATION OF RESULTS**

The internal control produces a 434 bp PCR product. The specific products for the reactions are all smaller than this internal control product. Rare Screen tubes can produce more than one specific product (multiplex reactions), depending on which of the rare alleles are present. The sizes for all specific products from the reactions are listed on the Recording Sheet for the kit (Rare ID only).

For each sample note the tubes with allele-specific PCR products on the appropriate (Rare Screen, Rare ID) Recording Sheet, using the row or table provided. For Rare ID use the patterns on the Results Table to determine the alleles present in the sample. For Rare Screen the appearance of a band indicates the presence of one of the 5 rare alleles detected by the assay (Kp<sup>a</sup>, Lu<sup>a</sup>, Yt<sup>b</sup>, Co<sup>b</sup>, Kn<sup>b</sup>). Rare alleles must be further identified by running the Rare ID assay on the sample. Special attention should be paid to the performance notes at the bottom of each Results Table; these provide useful information to aid in the interpretation of band patterns.

## LIMITATIONS

- Red Cell EZ TYPE® Rare ID kits contain materials for the amplification of sequences in genomic DNA by the polymerase chain reaction (PCR) and subsequent determination of Kp<sup>a/b</sup>, Lu<sup>a/b</sup>, Di<sup>a/b</sup>, Wr<sup>a/b</sup>, Co<sup>a/b</sup>, Yt<sup>a/b</sup>, Kn<sup>a/b</sup>, Do<sup>a/b</sup> alleles, as noted on the specific kit's Recording Sheet.  
Red Cell EZ TYPE® Rare Screen contains materials for the amplification of sequences specific for Kp<sup>a</sup>, Lu<sup>a</sup>, Yt<sup>b</sup>, Co<sup>b</sup>, or Kn<sup>b</sup> from genomic DNA. Other variations, including unknown variants in the relevant gene sequences, may adversely affect results.
- The determination of specific alleles by Red Cell EZ TYPE® Rare Screen and Rare ID are intended for use as an adjunct to serologic typing, and cannot be used to replace serologic testing.
- To ensure optimal results in the Red Cell EZ TYPE® assay, DNA samples should have a concentration between 25 ng/μL and 100 ng/μL with a 260 nm/280 nm ratio of 1.60 – 2.0. Poor amplification may result from insufficient DNA added to the PCR reaction, or from poorly-purified sample DNA.
- False-negative results may stem from improperly handled specimens, procedural errors, amplification inhibitors, or from poorly-purified DNA.
- False negative results may stem from use of a DNA polymerase enzyme other than those listed in **Materials Required** (but not provided).
- Contamination from carryover nucleic acids, excessive genomic DNA loaded to amplifications, or failure to use indicated temperatures may result in false-positive amplifications.
- The thermal cycler program has been tested with the thermal cyclers PE 9600 and PE 9700 (all Perkin Elmer/Applied Biosystems), the PTC-100 and PTC-200 (BioRad). Some adjustments to the indicated cycling parameters may be required for use of the assay in other thermal cyclers.
- Thermal cyclers should be calibrated and maintained according to the manufacturer's instructions.

## REFERENCES

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3. **Crew VK:** Molecular bases of the antigens of the Lutheran blood group system. **Transfusion** 2003, v43: 1729 – 1737



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**Red Cell EZ TYPE®**  
**(Rare ID, Rare Screen)**

### *RESEARCH USE ONLY*

- STORE AT ≤ -20°C for Kit
- STORE AT 15 to 30°C for E-Gels