











PRODUCT INSERT

LIFECODES RBC TYPING KIT for use with Luminex®

For Research Use Only, Not for In Vitro Diagnostic Use

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DEFINITION OF SYMBOLS (Product Labels and Supplemental Documents)

Lot Number		Catalog Number	REF	Temperature limitations		Upper limit of temperature	
Expiration Date	 (exp)	Keep away from light		Sufficient for N tests		Do Not Freeze	
Caution – See instructions for use		See instructions for use		Manufacturer			

PRODUCT DETAILS

LIFECODES RBC Typing Kit:

The LIFECODES-RBC assay uses single nucleotide polymorphism (SNP) detection by the Polymerase Chain Reaction – Reverse Sequence Specific Oligonucleotide (PCR-RSSO) method. This multiplex assay has been designed to genotype the antigens of the following highly prevalent blood groups:

Kell – K, k, Kpa, Kpb, Kpc, Jsa, Jsb

Kidd – Jka, Jkb, Jk (Finnish null)


Duffy – Fya, Fyb, Fyx (a-b weak), FyGATAsil

MNS – M, N, S, s, S-s-Uvar (includes 2 S silencing mutations: –GPB230 and GPB intron +5)

Rh – C, c, E, e

Dombrock – Doa, Dob

REAGENTS BY PRODUCT NUMBER

RBC LIFECODES RBC Typing Kit for use with Luminex					Product # 728100-50
Reagent	Product Number	Fill volume	Storage	 50 Sufficient for 50 samples	
MX-RBC	LIFECODES RBC Master Mix	728102	580 µL		2 to 8°C
BM-RBC	LIFECODES RBC Probe Mix †	728101	810 µL		2 to 8°C Protect From Light
DS	Dilution Solution	628515	19.7 mL		18 to 30°C

†Probe Mixes are light sensitive: keep away from light.

CAUTION: Do not freeze components and Do not use components past their expiration dates.

MATERIALS, REAGENTS, AND EQUIPMENT REQUIRED BUT NOT PROVIDED (as listed or equivalent)

Reagents or Chemicals or Kits

- R-Phycoerythrin Conjugated Streptavidin (SA-PE), 1mg/mL (Lifecodes, Cat. No. 628511)
- Recombinant *Taq* Polymerase (Lifecodes, Cat. No. 167075)
- 70% Isopropanol or 20% household bleach

Ancillaries

- PCR tubes and caps
- Corning: #6542 (VWR #29442-354) - 0.2mL 8 strip tubes and caps **OR**
- ABGene/ThermoFast 96 well PCR plates, skirted, cat# AB-1000, Natural, 50 plates/box
- Thermal cycler (PCR) 96 well plates (Costar®, Cat. No. 6509)
- Microseal™ Film (Bio-Rad, Cat. No. MSA-5001) or Tape (Costar®, Cat. No. 6524)
- Barrier filter tips
- Pipettors, multichannel pipettors and tips (1-20µL, 20-200µL, 1000µL)
- MATCH IT!-RBC Software (Supplied; Lifecodes, Cat. No. 888729)
- MATCH IT!-RBC - Lot Information Update CD (Supplied; Lifecodes, Cat. No. 888728)

Instruments and Related Ancillaries

- Luminex® Instrument and XY Platform (Lifecodes, Cat. No. 888300)
- Luminex Sheath Fluid (Lifecodes, Cat. No. 628005)
- Luminex Calibration beads
 - Cal 1 (Lifecodes, Cat. No. 628006)
 - Cal 2 (Lifecodes, Cat. No. 628007)
 - Con 1 (Lifecodes, Cat. No. 628008)
 - Con 2 (Lifecodes, Cat. No. 628009)
- Thermal cycler (Required Specifications: heated lid, adjustable ramp times, minimal thermal range 2 to 100°C, accuracy within ± 0.5°C)
- Vortex mixer
- Bath sonicator (Fisher Scientific, Cat. No. FS15 or FS20)
- Microcentrifuge
- Heat Block (Fisher Scientific Standard Heatblock, Cat. No. 13259-030)
- Rubber mat or metal plate

REAGENT STORAGE AND USE

1. Refer to kit component packaging label for proper storage temperatures.
2. If salts have precipitated out of solution during shipping or storage, resolubilize completely prior to use by vortexing at room temperature (18 to 30°C).
3. Probe mixes and R-Phycoerythrin Conjugated Streptavidin are light sensitive. **Keep away from light. Do not freeze these reagents.**
4. Do not use R-Phycoerythrin Conjugated Streptavidin that has been frozen during shipment or storage.
5. Do not use components past their expiration dates.

GENOMIC DNA PREPARATION AND USE

1. DNA can be prepared using any preferred method. The assay has been tested on genomic DNA from whole blood.
CAUTION: All biological and blood samples should be treated as potentially infectious. **Use Universal Precautions when handling.**
2. DNA extracted from blood preserved in heparin cannot be used in this assay.
3. The presence of alcohol, detergents or salts may adversely affect DNA amplification.
4. Absorbance measurements of the DNA sample should yield a 260nm to 280nm ratio ranging from 1.65 to 2.0.
5. The isolated DNA should be resuspended and stored in 10mM TRIS, pH 8.0 to 9.0, or in nuclease free water. If a chelating agent such as EDTA is present, the final concentration of the chelating agent should not exceed 0.5mM.
6. Final DNA concentration should be 10 to 200 ng/μL.
7. DNA can be used immediately after isolation or stored at -20°C for up to 1 year. Repeated freeze/thawing should be avoided **since this can result in DNA degradation.**

WARNING AND PRECAUTIONS

1. For Research Use Only – Not for in vitro diagnostic use.
2. Separate pipettes should be designated for Pre-PCR manipulations as well as for Post-PCR manipulations.
3. All biological and blood samples should be treated as potentially infectious. **Use Universal Precautions when handling.**
4. Dilution Solution, Probe mixes, *Taq* Polymerase and R-Phycoerythrin Conjugated Streptavidin contain hazardous compounds. Avoid contact with skin and eyes and dispose of accordingly. See Material Safety Data Sheets for additional information (www.gen-probe.com).

PROCEDURE

A. Genomic DNA Purification:

1. See Genomic DNA Preparation and Use for important information.
2. Purify DNA using method of choice.
3. Final concentration of genomic DNA should be 10 to 200 ng/ul.
4. Adjust DNA concentrations, if necessary, using nuclease free water. Keep all samples at similar concentrations.

B. Amplification:

1. Bring Master Mix kit component to room temperature.
2. Prepare Master Mix reaction solution using the following table. Prepare immediately prior to use. Do not store. Total volume per reaction is 25µL. It is recommended that one water blank be run with each test.

<i>Component</i>	<i>Amount per PCR sample reaction</i>
Master Mix	10 µL
Genomic DNA	Total of ~100 ng
Taq polymerase	0.25 µL (1.25 U)
Nuclease-free water	To 25 µL final volume

3. Aliquot DNA (~100ng optimal; 40 to 200ng acceptable) into PCR tubes.
4. Aliquot Master Mix reaction solution into tubes. Discard any remaining Master Mix reaction solution.
5. Place samples in the thermal cycler and run amplification program. The program shown below has yielded optimal results in internal testing. Conditions may need to be altered in order to optimize thermal profiles for individual thermal cyclers used.

95° C for 4 min <u>Number of cycles: 1</u>
95° C for 30 sec 51° C for 45 sec 65° C for 2 min : 30 sec <u>Number of cycles: 45</u>
65° C for 5 min

C. Hybridization:

IMPORTANT NOTES:

- **Pre-warm Probe Mix.**
- **Keep beads protected from light.**
- **Vortex beads.**
- **Dilute all hybridized samples at 56°C before removing tray.**

1. Warm Probe Mix at 55° to 60°C for 5 to 10 minutes.
2. Sonicate and vortex Probe Mix briefly to thoroughly resuspend the beads.
3. Aliquot 15µL of Probe Mix into each well of a Costar plate (vortex Probe Mix after every 8 samples).
4. Aliquot 5µL of PCR product into each well.
5. Seal plate with polyethylene tape or film.
6. Place in thermal cycler and run hybridization program. Use a rubber mat or metal plate to prevent evaporation.

56°C for 22 min 56°C HOLD

- Prepare a mixture of Dilution Solution and PE-Streptavidin (1mg/ml) using the table shown following. Protect mixture from light.

# of Samples	Dilution Solution (DS)	SA-PE
1	170 µL	3.4 µL
5	850 µL	17.0 µL
10	1700 µL	34.0 µL
20	3400 µL	68.0 µL
50	8500 µL	170.0 µL

- At the 56°C HOLD step, dilute each sample with 170µL of the Dilution Solution/PE-Streptavidin mixture.
NOTE: Do not remove the tray from the thermal cycler until all samples have been diluted.
- Remove the sample tray from the thermal cycler and immediately place in the Luminex instrument for analysis.
NOTE: For best results, acquisition should begin within 5 minutes after hybridization program ends.

D. Analysis Using Luminex Instrument (refer to Luminex IS User's Manual for instrument operation, including daily startup, calibration, maintenance, and shutdown procedures):

- Turn on the Luminex Instrument between 30 minutes and 4 hours before assaying the samples.
- Import the lot specific IDT template provided on the "RBC Lot Information Update CD" using the Luminex IS User's Manual. Please note that the template versions are lot number specific and correspond to the kit lot numbers.
- Prior to analyzing the samples on the Luminex Instrument, set up a Batch Run by which the samples will be analyzed. For example, if analyzing an RBC run, add a Batch Run for RBC.
 - Select **Create a New Batch** from the File menu.
 - Follow the stepwise instructions that appear on the screen for creating batches.
 - When naming the batch, do not include commas in the name; information located after a comma will be lost in data export.
 - For further instructions on creating batches and multibatches, refer to the Luminex IS User's Manual.
- Click the **Eject** icon to eject the plate holder. Place the 96 well thermal cycler plate containing the samples in the XYP heater block present on the plate holder.
- Click the **Retract** icon. The samples are now ready to be analyzed. An instrument priming step should be performed before starting the run.
- After the samples have been run through the instrument, a sanitization step with 70% Isopropanol or 20% household bleach should be performed followed by two wash steps. The instrument can be turned off at this point if it is not going to be used for the remainder of the day.
- After a batch is complete, the data are exported as a Comma Separated Values (csv) file. These files are named 'OUTPUT.CSV' and saved in a folder with the Batch Name entered into the Luminex IS. These data are then available for analysis using the MATCH IT!-RBC software.

E. Analysis Using Lifecodes MATCH IT!-RBC Software:

- Import the lot specific EDS file provided on the "RBC Lot Information Update CD" using the **Lot Data Set Import** tab.
- Import the csv file obtained from Luminex analysis using the **Batch Data Import** tab.
- Analyze the batch using the User's Manual for MATCH IT!-RBC Software.
- See User's Manual for MATCH IT!-RBC Software User's Manual for a description of possible run results.
 - Indeterminate results are noted by an "ID" label in the software report.
 - Indeterminate (ID) results can occur due to low amplification, probe value too close to the cut off, or an unexpected rare result. In these cases, the sample should be repeated or typed using another red cell typing technology.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE		SOLUTION
Low Bead Count	Probe Mix not well suspended		Prewarm, sonicate, and vortex probe mix and repeat assay.
	Instrument not functioning properly	Out of Calibration	Calibrate instrument (<i>refer to Luminex IS User's Manual</i>).
		Sample flow path blocked	Remove and sonicate needle. Perform backflush. Call Lifecodes Technical Support if problem persists (888-329-0255).
"Failed" sample	Sample failed to amplify or amplified poorly	Low DNA	Check DNA concentration and purity (see Genomic DNA Preparation and Use).
		Salts in Master Mix are out of solution	Heat Master Mix at 37°C for 5 minutes, vortex gently, and spin down briefly (2 to 5 secs).
		Poor <i>Taq</i> Polymerase	Use only Recombinant <i>Taq</i> . Lifecodes Cat. No. 167075 is recommended.
	Amplification conditions not within specific parameters		Run thermal profile on thermal cycler to verify parameters are within specified parameters. Adjust if necessary.
	Low Median Fluorescent Intensity (MFI) value		Ensure Dilution Solution is stored at room temperature (18 to 30 °C).
			Warm Dilution Solution at 45°C for 5 minutes before use and vortex.
			Replace R-Phycoerythrin Conjugated Streptavidin.
	Evaporation during hybridization		Ensure the plate is sealed tightly.
If not using an entire plate, leave one row empty on each side of samples to be assayed to assist in obtaining a tight plate seal.			

SPECIFIC PERFORMANCE CHARACTERISTICS

Specific performance characteristics have not been determined.

MANUFACTURER

Manufacturer: Gen-Probe Transplant Diagnostics, Inc.,
550 West Avenue, Stamford, CT 06902.
Phone: 203-328-9500, 888-329-0255
Fax: 203-328-9599

European Technical Service: Phone: +32/3 385 4791