EN

Instructions for Use

ERY Q Kits

CE IVD

Test kit for the determination of blood groups, HNA and HPA specificities
on a molecular genetic basis

REF	728401	ERY Q Weak D
REF	728402	ERY Q HPA
REF	728403	ERY Q Partial D
REF	728404	ERY Q HNA
REF	728405	ERY Q RH

Contents

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1. Intended use

The ERY Q kits are intended for typing of blood group, platelet and granulocyte characteristics using genomic DNA samples from donors, recipients and pregnant women. They are designed for use by trained qualified personnel in specialised laboratories. The molecular genetic typing is carried out using the SSP PCR technique and real-time detection (Realtime PCR) of the amplicons.

The ERY Q RH, -Partial D and Weak D kits are intended exclusively for the second line determination of RH, Partial D and Weak D characteristics. They are used to complement and confirm serological preliminary findings in case of discrepant or doubtful typing results.

For the determination of HPA and HNA characteristics with the ERY Q HPA and -HNA kits, an initial serological typing is not necessarily required.

2. Product description

The ERY Q Kits are used for the molecular genetic determination of blood group, HNA and HPA alleles. All clinically relevant alleles are covered, see chapter 8 - kit specificities. The ERY Q typing kits contain all components required for the PCR reaction. The evaluation is done with the PlexTyper software.

3. Test principle

The test is performed with genomic DNA as starting material. The DNA is amplified in a PCR with sequence-specific primers (SSP). The primers were specially developed for the selective amplification of segments of specific alleles or allele groups. The amplicons are detected (real-time PCR, RT-PCR) with likewise gene locus specific fluorescence dye-labelled hydrolysis probes (TaqMan® probes), which increases the sensitivity and specificity of the test compared to a conventional gel-based SSP. If amplicons are present, the probes are hydrolysed by the Taq polymerase and a fluorescence signal is generated which increases proportionally to the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the RT-PCR cycler. An internal amplification control (human HGH gene) is included in the multiplex PCR reaction which is detected in a different colour channel than the specific reactions.

4. Material

4.1 Content of the kits

- **260 µl Plex Mix**, ready to use, contains dNTPs, Taq Polymerase, reaction buffer
- **12 x ERY Q 8-well PCR strips** for the molecular genetic determination of blood group, HNA or HPA alleles. The pre-dropped and dried reaction mixes contain specific primers and probes as well as HGH-specific control primers and probes (oligomixes)
- **12 x PCR Caps (á 8)**

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4.2 Additionally required reagents and devices

- Reagents for DNA isolation (validated extraction kits see 6.2)
- RT-PCR Cycler (validated cycler see 4.3)
- Aqua dest.
- Variable pipettes (0.5 - 1000 µl) and pipette tips

4.3 Validated RT-PCR cycler

Bio-Rad: CFX96 Touch™ Real-Time PCR Detection System

Following fluorophores are used for the ERY Q product line.

Fluorophore	Wave length in nm
FAM	Excitation: 495 Emission: 520
CAL Fluor® Orange 560	Excitation: 538 Emission: 559
CAL Fluor® Red 610	Excitation: 590 Emission: 610
Quasar® 670	Excitation: 647 Emission: 670

5. Storage and stability

All reagents must be stored at ≤ -20°C in temperature-controlled devices. The kits are shipped with ice packs. The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. The freeze-thaw cycle testing of the Plex Mix has shown that up to 6 cycles have no detrimental effects on the quality. It is recommended to aliquot the Plex Mix if required.

6. Test procedure

6.1 Precautions and special Remarks

Molecular genetic techniques are particularly sensitive methods and should only be performed by qualified personnel with experience in molecular genetic techniques.

Special precautions must be followed to avoid contamination and thus false reactions:

- Principally wear gloves during work (preferably powder-free).
- Use new tips with each pipetting step (with filter insert or integrated stamp).
- Work in two separate areas for pre-amplification (DNA-isolation, preparation of the reactions) and post-amplification (detection); use two separate rooms if possible.
- Use devices and other materials only at the respective workplaces and do not exchange them.

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6.2 DNA Isolation

The specimen material for the isolation of the genomic DNA must be sent in appropriate collection systems. The test requires EDTA or Citrate blood. The presence of Heparin may potentially inhibit the PCR reaction (1), therefore such collection systems are not suitable and must not be used.

Recommended DNA-Extraction Kits:

- Qiagen QIAamp DNA Blood Kits (columns)

Manual isolation or automated DNA isolation (QIAcube) are suitable.

If the standard method established in the laboratory is to be applied for isolation of gDNA without using the specified test kit, it must be validated by the user.

The purity indices must be in the following range:

- OD_{260} / OD_{280} = > 1,5 and < 2,0
  Higher values are an indicator for the presence of RNA, lower values indicate protein contamination.
- OD_{260} / OD_{230} = > 1,8
  Lower values indicate contamination with carbohydrates, salts or organic solvents.

6.3 Amplification

Note:

- The reaction volume for each RT-PCR-preparation is 10 µl (each well).
- For the test a DNA concentration between 10 – 30 ng/µl is required.

Pipetting Process:

For one well, pipette 2 µl Plex Mix, 1 µl DNA specimens and 7 µl Aqua dest. into the reaction tube.

For each specimen (8 well strip) a pre-mix is created (a 9-fold preparation is recommended).

18 µl Plex Mix
9 µl Specimen DNA
63 µl Aqua. dest.

From this pre-mix 10 µl are dispensed into each of the 8 wells.

If a **negative control (NTC)** should be performed, prepare a test with Aqua dest. instead of DNA specimen.

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Note: When pipetting into the PCR wells it is important not to allow the pipette tip to contact the dried mix (dyed blue) in the bottom of the well. It is advisable to pipette to the side of the well, see figure 1.

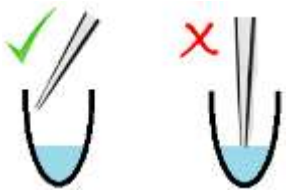

Figure 1: Schematic description of the pipetting procedure

Close the reaction tubes and briefly spin the liquid down. Make sure that the strips are **completely closed** by the caps. Make sure that there are **no bubbles** in the reaction tubes. If bubbles appear, gently tap the tubes on the laboratory bench to remove them.

Then perform the PCR reaction using the following program:

Table: PCR-Program

Program Step	Time [s]	Temperature [°C]	Ramp rate [°C/s]	Plate read	Number of Cycles
Initial Activation	120	96	2,5	-	1
Denaturation	5	98	2,5	-	13
Annealing + Extension	25	68	2,2	-	
Denaturation	5	98	2,5	-	37
Annealing + Extension	25	68	-	yes	

Note: With the CFX96 Touch™ Real-Time PCR Detection System, a modified heating rate of the device (ramp rate) must be used. These are listed in the PCR program table above ("Ramp rate" column).

Other thermocyclers are not validated and may require different PCR parameters. PlexTyper software is essential for interpretation of results and the software will only import data from validated instruments.

6.4 Evaluation and Interpretation of the Results

For evaluation and interpretation of the data it is required to use the PlexTyper software (available free of charge from BAG Diagnostics) in conjunction with PlexTyper kit specific data files. The kit files required for the evaluation are available from the BAG Diagnostics download server (www.service.bag-diagnostics.com).

Please make a note of the product and lot number of the kit used. The kit files are product and lot specific. Use of incorrect kit files could result in incorrect genotyping.

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For interpretation of the results from a thermal cycler the data must be transferred (e.g. with a suitable USB drive) to a computer running BAG Diagnostics PlexTyper Software. Please use the PlexTyper instructions for use for interpretation of the data.

It is possible, but not essential to perform a broad review of the data on the thermocycler software. For example, valid amplification must show suitable fluorescence signals for the internal amplification control in the FAM channel. Positive reactions show a positive colour signal in the corresponding colour channel.

A negative control (NTC) is used as contamination control. If DNA or contaminating amplicon is inadvertently added to the NTC reaction a positive signal will occur. If the Cq is less than 36 it will be detected as possible contamination by the PlexTyper software and a warning message is generated. Amplification signals above Cq 36 in the NTC are regarded as PCR artefacts and are disregarded. If PCR contamination is suspected, it is advisable to follow local decontamination guidelines and to exchange the reagents.

The raw data collected from the cycler-specific software will be imported into the PlexTyper software. Based on the Cq values, RFUs (Relative Fluorescence Units) and the shape of the amplification curve the PlexTyper software determines the positive and negative reactions from which the molecular genetic blood group type, or HNA / HPA specificities of the specimen are determined

7. Warnings and disposal instructions

The kits should only be used by specially trained, qualified personnel. All work should be performed in accordance with Good Laboratory Practice.

All materials of biological origin used in the test to obtain DNA (e.g. blood) should be considered as potentially infectious. Therefore, appropriate safety precautions are recommended when handling biological materials (do not pipette by mouth; wear protective gloves when performing the test; disinfect hands after performing the test).

Biological materials must be inactivated before disposal (e.g. by autoclaving). Disposable materials must be autoclaved or incinerated after use.

Spilled potentially infectious material should be removed immediately with an absorbent paper towel and the contaminated area disinfected with an appropriate disinfectant or 70% Ethanol. Material used to remove spills must be inactivated before disposal (e.g. by autoclaving).

Disposal of all specimens, unused reagents and waste should be in accordance with the legislation of the respective country and the local authorities.

Microbial contamination of reagents while taking aliquots should be avoided. The use of sterile disposable pipettes and pipette tips is recommended. Do not use reagents looking cloudy or showing signs of microbial contamination.

A Material Safety Data Sheet (MSDS) respectively a declaration on Material Safety Data Sheets is available for download at **www.bag-diagnostics.com**.

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8. Kit specificities

The combination of primers and probes allows a determination of human blood group, HNA and HPA alleles according to the lot-specific data. The accuracy and reproducibility of the reactivity of the test kit will be checked for each lot with control specimens with known genotypes.

Product	Detection of the following blood group, HNA and HPA characteristics	
ERY Q HPA REF 728402	HPA 1a / 1 b HPA 2a / 2 b HPA 3a / 3b HPA 4a / 4b HPA 5a / 5b HPA 6a / 6b HPA 9a / 9b HPA 15a / 15b	
ERY Q HNA REF 728404	HNA 1a / 1b / 1c HNA 2 (*787A) / 2null (*787T) HNA 3a / 3a var./b HNA 4a / 4b HNA 5a / 5b	
ERY Q RH REF 728405	RHD*01 (DD) RHD*01N.01 (dd) RHD*DEL1 (K409K) RHD*11 (M295I) RHD* DEL8 (IVS3+1G>A) RHD*08N.01 (Psi/Ψ) RHD-CE (8-9)-D RHD*01N.08 (W16X)	RHCE*C RHCE*C^{W} RHCE*E RHCE*e RHCE*c
ERY Q Weak D REF 728401	RHD*01W.1.1 RHD*01W.1 RHD*01W.2 RHD*01W.3 RHD*01W.38 RHD*01W.5 RHD*01W.17 RHD*15 RHD*01W.20	RHD*01W.31 RHD*09.01.00 RHD*01EL.01 RHD*01EL.08 RHD*09.05 RHD*08N.01 RHD*01W.14 RHD*11 RHD*09.03.01,09.04

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Product	Detection of the following blood group, HNA and HPA characteristics	
ERY Q Partial D REF 728403	RHD*01	RHD*10.04, *10.05., *10.05.01
	RHD*01N.01	RHD*14.01
	RHD*02	RHD*14.02
	RHD*03.01,03.03	RHD*17.01 - *17.03
	RHD*03.02	RHD*17.05
	RHD*04.01	RHD*08N.01
	RHD*04.06, *04.05	RHD*19
	RHD*04.03	RHD*25
	RHD*04.04	RHCE*01.22
	RHD*05.01-*05.10 RHD*13.01,*13.02	RHD*01N.02
	RHD*06.01	RHD*01N.03
	RHD*06.02	RHD*01N.04
	RHD*06.03	RHD*01N.05
	RHD*06.04	RHD*01N.07
	RHD*07.01, 07.02	RHD*D-CE(8-9)-D
	RHD*09.01.00	RHD(delEx9)
	RHD*09.02.00, *09.02.01	RHD*D-CE(10)
	RHD*10.00-*10.03, *10.06 - *10.15	RHD*01N.09

9. Performance characteristics

For the ERY Q HPA, HNA, RH, Weak D und Partial D kits, performance studies were conducted with pretyped DNA samples. The typing results were compared with results obtained with CE-certified typing reagents (e.g. SSP, serology) and nucleic acid sequencing.

If DNA samples for rare alleles were not available, these were replaced by synthetically produced DNA samples and the reactivity of the mixes was tested.

For the products external and internal performance evaluation studies were performed in various blood donation centres, medical laboratories and at BAG by qualified personnel. The typing with the ERY Q kits resulted in the following conformity to the pre-typing results:

Kit	Amount of tested samples	Accordance to the reference typing
ERY Q HPA	116	100%
ERY Q HNA	80	100%
ERY Q RH	200	100%
ERY Q Weak D	200	100%
ERY Q Partial D	200	100%

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10. Limits of the method

If no clear result is obtained with the ERY Q kits (e.g. due to unknown alleles which are not detected with the existing primers and probes), national transfusion guidelines should be followed in accordance with the serological typings. Sequencing of samples with unclear results to clarify the genotype is recommended. The test results should be evaluated taking into account the genetic variance in different ethnic groups. In case of doubt the phenotype is valid.

Since the RT-PCR method is very sensitive to cross-contamination with DNA, this must be taken into account during isolation. Special care should be taken to avoid contamination of kit reagents and other laboratory materials with amplicons or DNA.

It is recommended to perform a negative control with Aqua dest.. No fluorescence signal with a Cq < 36 should be detected in the negative control with Aqua dest.. In the case of signal development in the negative control, the PCR laboratory workplace may need to be decontaminated from DNA and the reagents exchanged if necessary.

All devices (e.g. pipettes, real-time cyclers) must be calibrated according to the manufacturer's specifications.

11. Internal quality control

Internal quality controls for new lots can be performed using a combination of DNA specimens with known genotype. An internal amplification control (IAC) to verify successful amplification is included in the dried oligomixes.

Performance of negative controls to detect possible contaminations is recommended. For this purpose, prepare a test without DNA (NTC), see chapter 6.3. Amplification.

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12. Troubleshooting

Symptom	Possible Reason	Potential Solution
Poor or no Signal	Presence of an inhibitor.	Use fresh reagents.
	No gDNA in the reaction.	Repeat test. Pay attention to correct pipetting.
	Wrong amplification parameters.	Check PCR program and ramp rate.
	Contaminated or degraded DNA.	Check concentration / quality of DNA. Check DNA on a gel. Repeat DNA isolation.
	Degraded Fluorescent probes or primers.	Use new ERY Q kit. Avoid exposure to light and frequent thawing and freezing. Pay attention to storage conditions.
	Bubbles in the PCR reaction / residual liquid at the inner wall of the tube.	Careful pipetting. Spin down PCR plate.
	Evaporation of the reagents due to incorrect closing of the PCR tubes.	Make sure that the PCR tubes are closed properly. Caution with adhesive foils in the edge area.
Signal in the Negative Control	Contamination with DNA in the negative control.	Repeat the negative control. Decontaminate the workplace.

13. Trade names used

TaqMan® is a tradename of Roche Molecular Systems Inc.

® Cal Fluor & Quasar Dyes are the registered trademark of LGC Biosearch Technologies

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14. Explanation of the symbols used on the labels

	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Consult instructions for use
	Use by
	Manufacturer
eIFU | VXX/XXXX	Electronic instructions for use Version of the actual instruction for use
IVD	For in vitro diagnostic use
LOT	Batch code
CONT	Content, contains
BLOOD TYPING	Intended Use: Blood group typing
HNA TYPING	Intended Use: Determination of HNA specificities
HPA TYPING	Intended Use: Determination of HPA specificities
REF	Catalogue number
PCRSTRIP	PCR strips
REACTIONMIX	Reaction mixes
PCRCAP	PCR caps
RTU	Ready to use
PLEX MIX	Mastermix, contains dNTPs, Taq Polymerase, reaction buffer

15. Literature

1. Beutler, E. et al., 1990. BioTechniques 9:166

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