EN

Instructions for Use

ERY Q® Kits

CE IVD

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
REF	728406	ERY Q® ABO
REF	728407	ERY Q® KKD/MNS
REF	728408	ERY Q® Rare
REF	728409	ERY Q® ABO variant

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1. Intended use

The ERY Q® kits are in vitro diagnostic medical devices for use by professionals in specialised laboratories.

The ERY Q® RH, -Partial D, -Weak D, -ABO, -ABO variant and -KKD/MNS kits are intended for second line determination of blood group characteristics using genomic DNA samples from donors, recipients and pregnant women. The molecular genetic second line determination is carried out using the SSP PCR technique and real-time detection (Realtime PCR) of the amplicons.

The ERY Q® RH, -Partial D, -Weak D, -ABO und -ABO variant kits must be used exclusively for the second line determination of the respective characteristics. They are used to complement and confirm previous serological findings in case of discrepant or doubtful typing results. The same applies to the determination of Kell (K), Kidd (K) and Duffy (D) characteristics. The test system for the determination of KKD characteristics must only be used for second line determination.

The ERY Q® HPA, -HNA and -Rare kits are intended for typing of blood group, platelet and granulocyte characteristics using genomic DNA samples from donors, recipients and pregnant women. The molecular genetic typing is carried out using the SSP PCR technique and real-time detection (Realtime PCR) of the amplicons.

For the determination of MNS characteristics using the ERY Q® KKD/MNS Kit and for genotyping of HNA, HPA and rare blood group characteristics using the ERY Q® HPA, -HNA and -Rare Kits, an initial serological pre-typing is not mandatory.

2. Product description

The ERY Q® Kits are used for the molecular genetic second line determination/ 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.

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4. Material

4.1 Content of the kits

- **Plex Mix**, ready to use, contains dNTPs, Taq Polymerase, reaction buffer
- **ERY Q® 8-well PCR strips** with pre-dropped and dried reaction mixes containing specific primers and probes as well as HGH-specific control primers and probes (oligomixes).
- **PCR Caps (á 8)**

The Plex Mix is also available separately as an IVD accessory (REF 728298). The Plex Mix is only to be used for the BAG products specified in the Plex Mix Instructions for Use.

4.2 Additionally required reagents and devices

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

Plate/Stripe Adapter:

- For LightCycler® 480 II: Vari-Plate™ 96 Well Semi-Skirted Frame, Roche Style (Brooks Life Science (4titude), Order No. 4ti-0950W-F) or LightCycler® 8-Tube-Strip Adapter Plate (Roche, Order No. 06612598001)
- For QuantStudio™ 6 Flex System: Fast 96 Well Plate Adapter (Standard accessory, Order No. 4459846 for spare part, ThermoFischer (Applied Biosystem))

4.3 Validated RT-PCR cyclers

The following cyclers are validated for the use with the ERY Q® kits:

- CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad
- LightCycler® 480 II Real-Time PCR Detection System, Roche Molecular Systems Inc.
- QuantStudio™ 6 Flex System, Applied Biosystems

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 12 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.

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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.

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.

Validated DNA-Extraction Kits:

- Qiagen QIAamp DNA Blood Kits (columns)

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

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 Entering sample information into Plextyper Software

It is mandatory to use PlexTyper® Software to analyse ERY Q® data. It is advisable to enter the sample information into PlexTyper® and to prepare the test setup before setting up the PCR amplification to obtain the unique RUN ID. For detailed information about the PlexTyper® software please find the instruction for use of the PlexTyper® software on our website.
http://www.bag-diagnostics.com

6.4 PCR Setup

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.

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Pipetting Process:

The upper side of the strips is marked by the batch imprint, i.e. Mix 1 is located in the well below the batch imprint. Pay attention to the orientation of the strips, especially when loading the cycler.

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.

Note: For each sample of the ERY Q® ABO variant kit (2 x 8 well strips, which are marked with 1 and 2 behind the batch print) a pre-mix is prepared (an 18-fold preparation is recommended):

36 µl Plex Mix
18 µl Proben DNA
126 µl Aqua dest.

From this pre-mix 10 µl are dispensed into each of the 8 wells (or 16 wells).

For a negative control (NTC), prepare a test with Aqua dest. instead of DNA specimen.

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.

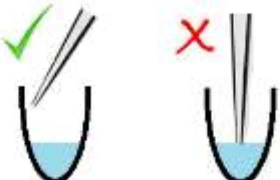

Figure 1: Schematic description of the pipetting procedure

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.

Note: Thermal cyclers other than those listed in point 4.3 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.

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6.5 Setup of the RT-PCR cyclers

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

6.5.1 CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad

Please note: Do not change the dye names in the CFX software. The PlexTyper® software needs the default dye names for evaluation and for a correct import.

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). Before starting the run **"All Channels"** must be selected and the lid temperature must be set to 105°C.

6.5.2 LightCycler® 480 II Real-Time PCR Detection System, Roche Molecular Systems Inc.

Please note that the light source for this cycler has been changed. From serial number 29001 it is a LED lamp, before there was a Xenon lamp. The test has been validated on an instrument with a LED lamp. It is expected that the older versions are compatible with the test as well, but it is likely that a colour compensation is necessary. Please contact BAG Diagnostics if you have an instrument with a Xenon lamp and your results are suboptimal.

Note: For the ERY Q® stripes a special holder for the Lightcycler® 480 II is required: Vari-Plate™ 96 Well Semi-Skirted Frame, Roche Style or LightCycler® 8-Tube-Strip Adapter Plate (see chapter 4.2). For more information please contact BAG Diagnostics.

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PCR-Program

Following the manufacturer's user guide for the LightCycler® 480 II, set up and save a PCR protocol with the following parameters:

Detection Format: ERY Q®, **Block size** 96, **Reaction volume** 10 µl

Step	Cycles	Analysis Mode	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp rate (°C/s)
Hold	1	None	96	None	00:02:00	2.5
Cycle	13	None	98	None	00:00:05	2.5
			68	None	00:00:25	2.2
Cycle	37	Quantification	98	None	00:00:05	2.5
			68	Single	00:00:25	2.2

Channels for LightCycler® 480 II Real-Time PCR Detection System

Use the following channel set up.

Excitation	Emission 488	510	580	610	640	660
440						
465		✓				
498						
533			✓	✓		
618						✓

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	FAM	1	10	1
533	580	O560 (CalFluor Orange560)	1	10	1
533	610	R610 (CalFluor Red610)	1	10	1
618	660	Q670 (Quasar670)	1	10	1

It is not necessary to utilise colour compensation facility in the LightCycler program as the PlexTyper® software performs these calculations during analysis.

Instrument Settings for Plate Type: White Plates

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6.5.3 QuantStudio™ 6 Flex System, Applied Biosystems

Note: For the ERY Q® stripes a special holder for the QuantStudioTM 6 Flex system is required: Fast 96 Well Plate Adapter (see chapter 4.2).
For more information please contact BAG Diagnostics.

Experiment Properties:

Instrument type:	QuantStudio™ 6 Flex System
Block type:	Fast 96-Well (0.1mL)
Experiment type:	Comparative Ct (ΔΔCt)
Reagent type:	TaqMan® Reagents
Run properties:	Standard

Please note: Custom dye calibration must be performed!

Define Targets:

Target Name	Reporter	Quencher	Color
FAM	FAM	NFQ-MGB	Green
Orange560	O560	NFQ-MGB	Orange
RED	R610	NFQ-MGB	Red
Q670	Q670	NFQ-MGB	Purple

Passive Reference: None

Assign: Assign all targets to each well.

Run Method:

Reaction volume: 10 µl

Stage	Cycles	Data Collection	Target (°C)	Hold (mm:ss)	Ramp rate (°C/s)
Hold Stage	1	Off	96	00:02:00	2.5
PCR Stage	13	Off	98	00:00:05	2.5
			68	00:00:25	2.2
PCR Stage	37	Off	98	00:00:05	2.5
		On	68	00:00:25	2.2

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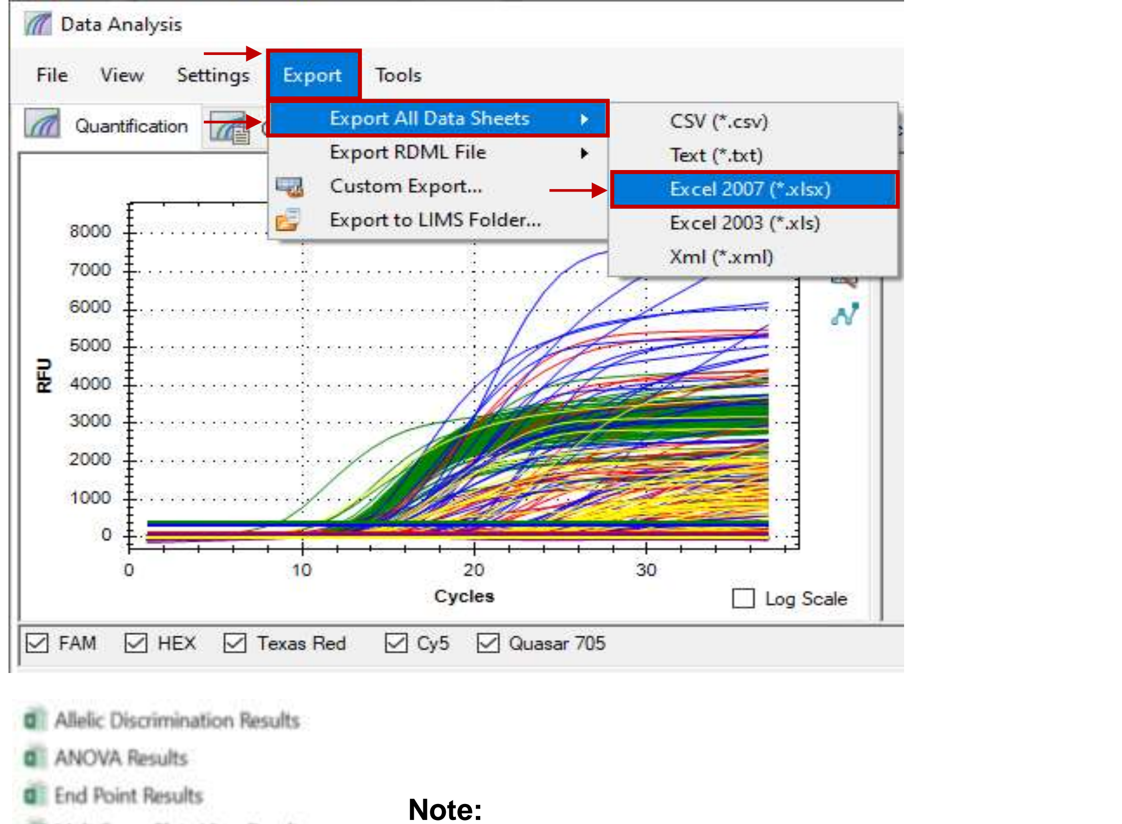
6.6 Export of the results

6.6.1 CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad

Open the data with the CFX software and then export the Excel 2007 (.xlsx) file.

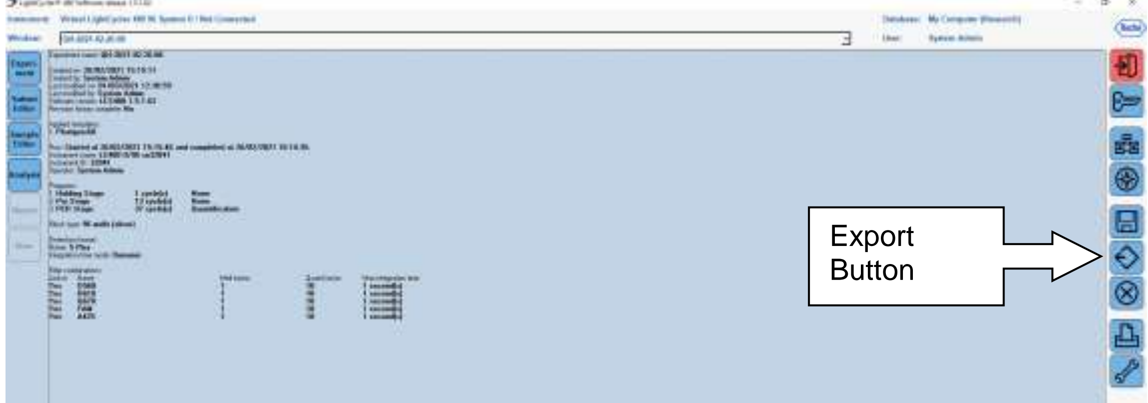


Allelic Discrimination Results
ANOVA Results
End Point Results
Melt Curve Plate View Results
Quantification Amplification Results
Quantification Cq Results
Quantification Plate View Results
Quantification Summary
Standard Curve Results

Note:
Only the file "Quantification Amplification Results" is needed. It is useful to delete the other files.

6.6.2 LightCycler® 480 II Real-Time PCR Detection System, Roche Molecular Systems Inc.

PlexTyper® utilises xml files from LightCycler® 480 II. After the run is complete there is no requirement for analysis in the Roche software. Export the raw data in XML form.



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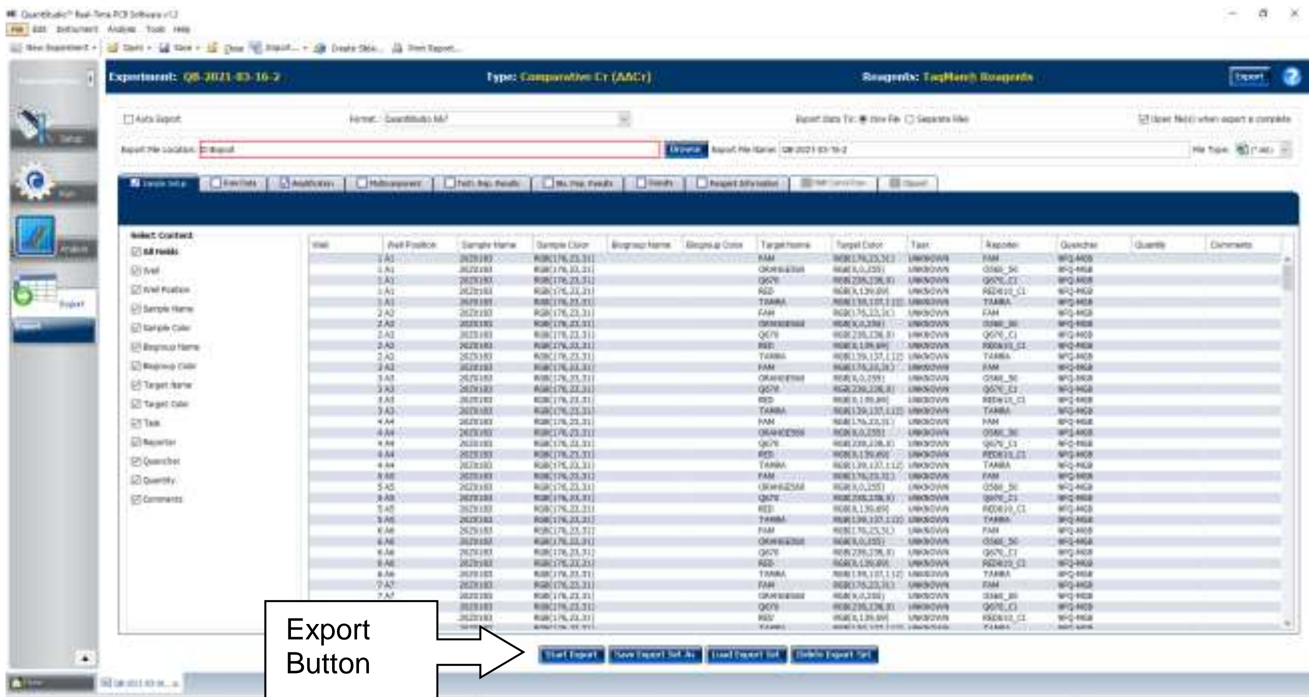
6.6.3 QuantStudio™ 6 Flex System, Applied Biosystems

Open the Export Menu and start the export of the “Sample Setup” and the “Amplification” tab as (*.xls) file.



6.7 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 and also specific for the RT-PCR cycler used. The use of incorrect kit files (false kit, false lot, false cycler) could result in incorrect genotyping.

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.

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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 chosen for the particular ERY Q® Kit allows the detection of the specificities listed below. The accuracy and reproducibility of the reactivity of the test kit will be checked for each lot with control specimens with known genotypes.

Product	Specificities	
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	Specificities	
ERY Q® Partial D REF 728403	RHD*01 RHD*01N.01 RHD*02 RHD*03.01,03.03 RHD*03.02 RHD*04.01 RHD*04.06, *04.05 RHD*04.03 RHD*04.04 RHD*05.01-*05.10 RHD*13.01,*13.02 RHD*06.01 RHD*06.02 RHD*06.03 RHD*06.04 RHD*07.01, 07.02 RHD*09.01.00 RHD*09.02.00, *09.02.01 RHD*10.00-*10.03, *10.06 - *10.15	RHD*10.04, *10.05., *10.05.01 RHD*14.01 RHD*14.02 RHD*17.01 - *17.03 RHD*17.05 RHD*08N.01 RHD*19 RHD*25 RHCE*01.22 RHD*01N.02 RHD*01N.03 RHD*01N.04 RHD*01N.05 RHD*01N.07 RHD*D-CE(8-9)-D RHD(delEx9) RHD*D-CE(10) RHD*01N.09
ERY Q® ABO REF 728406	ABO* A1.01 ABO* A2.01 ABO* B.01 ABO* O.01.01 ABO* O.02.01	
ERY Q® ABO variant REF 728409	ABO* A1.01 ABO* A2.01 ABO* A3.01 ABO* AEL.01 ABO* AW.04 ABO* AW.06 ABO* AW.07 ABO* AW.11 ABO* AW.30.01 ABO* cisAB.01	ABO* B.01 ABO* B3.02 ABO* BW.01 ABO* BW.09 ABO* O.01.01 ABO* O.01.02 ABO* O.02.01 ABO* O.01.57 ABO* O.04.01 ABO* O.04.02

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Product	Specificities	
ERY Q® KKD/MNS REF 728407	KEL*01.01 KEL*02 JK*01 JK*02 JK*01N.06# JK*02N.06# FY*01 FY*02	GYPA*01 GYPA*02 GYPB*03 GYPB*04 GYPB*03N.01# GYPB*03N.04# FY*02W.01 FY*02N.01
ERY Q® Rare REF 728408	KEL*02.03 KEL*02.04 KEL*02.06 KEL*02.07 DI*01 DI*02 DI*02.03# DI*02.04 DO*01 DO*02	LU*01 LU*02 CO*01.01 CO*02 YT*01 YT*02 VEL*01 VEL*−01 KN*01 KN*02

These specificities could not be tested positive due to their rarity.

9. Performance characteristics

For the ERY Q® HPA, HNA, RH, Weak D, Partial D, ABO, ABO variant, KKD/MNS and Rare 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	Number of tested samples with the Bio Rad CFX cycler	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%
ERY Q® ABO	92	100 %
ERY Q® ABO variant	116	100 %
ERY Q® KKD/MNS	82	*96,3 %
ERY Q® Rare	87	*97,7 %

* No clear sequencing result of the reference samples.

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Kit	Number of tested samples with the LightCycler® 480 II	Number of tested samples with the QuantStudio™ 6 Flex System	Accordance to the reference typing
ERY Q® HPA	10	12	100%
ERY Q® HNA	8	12	100%
ERY Q® RH	15	12	100%
ERY Q® Weak D	15	10	100%
ERY Q® Partial D	11	6	100%
ERY Q® ABO / ERY Q® ABO variant	14	12	100%
ERY Q® KKD/MNS	3	10	100%
ERY Q® Rare	11	12	100%

The specificity of the kits can be derived from the CFX validation, therefore a smaller number of samples is sufficient for the validation of further cyclers.

9.1 Cross reactive substances

Eight substances that might interfere with the assay were tested and the following concentrations were shown to have no detrimetal effect on the results:

Substance	Maximal non-inhibitory concentration
Protein (BSA)	0.2 mg/ml
TE (Tris/EDTA, pH 8.0)	7 mM Tris, 0.7 mM EDTA
NaCl	20 mM
Ethanol	1%
Haemoglobin	0.01 mg/ml
Sodium Citrate	7 mM
DNA extraction buffer 1 (Qiagen QIAmp DNA Blood Kits)	1%
DNA extraction buffer 2 (Qiagen QIAmp DNA Blood Kits)	2%

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.

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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.

To avoid false positive and negative reactions due to contamination, it is strongly recommended to carry out 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.

To avoid false positive and negative reactions due to contamination, it is strongly recommended to carry a negative control with Aqua dest.. For this purpose, prepare a test without DNA (NTC), see chapter 6.4 PCR Setup.

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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.	Repeat the negative control. If a signal is detected again, decontaminate the workplace and repeat the test.

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

Symbol	Explanation
Σ	Sufficient for n tests
(thermometer)	Storage temperature / Lower limit of temperature
(i)	Consult instructions for use
(hourglass)	Use by
(factory)	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	For blood group typing according to the intended use
HNA TYPING	For determination of HNA specificities
HPA TYPING	For determination of HPA specificities
REF	Catalogue number
PCRSTRIP	PCR strips
PCRSTRIP/1	PCR strips for mix 1- 8 of ERY Q® AB0 variant
PCRSTRIP/2	PCR strips for mix 9- 16 of ERY Q® AB0 variant
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

Find further information and instructions for use in other languages on our website http://www.bag-diagnostics.com or contact us directly at info@bag-diagnostics.com or phone: +49 (0)6404-925-125

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