

EN

Instructions for use

# ERY SPOT<sup>®</sup> SSO Kits

Electronic Instructions for use see [www.bag-healthcare.com](http://www.bag-healthcare.com)

Test kits for blood group typing on a molecular genetic basis

IVD

<b>REF</b> 728510: ERY SPOT <sup>®</sup> Common (96 Tests)	C €0123
<b>REF</b> 728511: ERY SPOT <sup>®</sup> Common (24 Tests)	C €0123
<b>REF</b> 728520: ERY SPOT <sup>®</sup> Rare (96 Tests)	C €
<b>REF</b> 728521: ERY SPOT <sup>®</sup> Rare (24 Tests)	C €
<b>REF</b> 728540: ERY SPOT <sup>®</sup> Weak D-TYPE (96 Tests)	C €0123
<b>REF</b> 728541: ERY SPOT <sup>®</sup> Weak D-TYPE (24 Tests)	C €0123
<b>REF</b> 726098: HISTO SPOT <sup>®</sup> Reagent Kit	C €

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## 1. PRODUCT DESCRIPTION

The ERY SPOT<sup>®</sup> SSO system is an in vitro diagnostic test for the specific and qualitative typing of the alleles of the Kell, Kidd, Duffy, MNS and rare blood group systems as well as the alleles of the Rhesus D system on a molecular genetic basis. The products are used to type blood group characteristics as well as to clarify, confirm and supplement serological findings using PCR (polymerase chain reaction) and hybridization of oligonucleotides (SSO technique).

The system consists of the ERY SPOT<sup>®</sup> typing kits, the HISTO SPOT<sup>®</sup> Reagent Kit, the MR.SPOT<sup>®</sup> processor and the HISTO MATCH ERY SPOT<sup>®</sup> Module interpretation software.

The ERY SPOT<sup>®</sup> typing kit contains all components required for the polymerase chain reaction (PCR) and testwells with immobilized sequence specific oligonucleotide probes for the detection of the PCR products. The HISTO SPOT<sup>®</sup> Reagent Kit contains the reagents for the hybridisation and detection of the PCR products. The MR.SPOT<sup>®</sup> processor is specifically designed to automate hybridization and detection. The MR.SPOT<sup>®</sup> processor can process 1-96 tests simultaneously. The HISTO MATCH ERY SPOT<sup>®</sup> Module software is required to interpret the results.

See Paragraph 14. Annex 1 for the list of alleles detected by the ERY SPOT<sup>®</sup> typing kits.

## 2. TEST PRINCIPLE

The test includes four basic steps:

- DNA isolation
- PCR amplification
- hybridization and detection
- data interpretation

DNA isolation is performed on the clinical sample, using the DNA isolation method established in the laboratory or using commercial kits. Then the DNA is amplified in a locus specific PCR reaction using the reagents (Primermix, Primer Solvent, Mastermix) provided with the kit. The specificity of the amplification is governed by a set of biotinylated primers that have been designed to uniquely amplify the chosen blood group allele. After the PCR amplification process, the PCR plate containing biotin labelled amplicon is transferred to the MR.SPOT<sup>®</sup> processor. MR.SPOT<sup>®</sup> adds hybridisation buffer to each well and then transfers the amplicon plus hybridisation buffer to a test well containing an array of immobilized sequence-specific oligonucleotide (SSO) probes. The biotin labelled amplicon binds to those SSO probes that contain a complementary target sequence and can then be detected by a colourimetric reaction. In order to prevent unspecific binding of the amplicon on the surface of the test wells they have been blocked with blocking buffer before transferring the amplicon.

After a stringent wash step to remove all unbound amplicon a streptavidin-alkaline phosphatase conjugate is added to the wells and binds to the biotin labelled amplicon captured by the SSO probe. After further wash steps, BCIP<sup>®</sup>/NBT substrate is added which produces a dark blue colour when converted by the alkaline phosphatase. The resulting coloured dots in the bottom of each test well are photographed by MR.SPOT<sup>®</sup> and the image is transferred into the HISTO MATCH ERY SPOT<sup>®</sup> Module software installed on the PC of the user. The image analysis program of the HISTO MATCH ERY SPOT<sup>®</sup> Module software determines the intensity of each spot in the array and compares it to the intensity of the background. From this data the positive and negative reactions are calculated. The pattern matching program of the HISTO MATCH ERY SPOT<sup>®</sup> Module software determines the blood group allele present in the sample based on the specific hybridisation pattern.

## 3. MATERIAL

### 3.1 Content of the ERY SPOT<sup>®</sup> typing kits

The content of the ERY SPOT<sup>®</sup> typing kits depends on the kits unit size. Please see table 1 for the detailed content of the ERY SPOT<sup>®</sup> typing kits.

**Table 1**

Component***	Description	Content 24 Tests	Content 96 Tests
<b>Testwells XXX</b>	<b>Testwells*</b> , individually packed, each strip containing 8 tests, contains immobilized, sequence specific oligonucleotide probes	3x	12x
<b>Primermix XXX</b>	<b>Primermix</b> , pre-dried, contains biotinylated primers for the chosen allele. The amount of dried primers in each tube is sufficient to perform 8 tests.	3x	12x
<b>SOLV PR XXX</b>	<b>Primer Solvent</b> , ready to use. The amount of primer solvent in each tube is sufficient for the resuspension of up to six dried primermix tubes.	1x	2x
<b>Mastermix ERY</b>	<b>Mastermix**</b> , ready to use, contains dNTPs, Taq polymerase, reaction buffer. The amount of mastermix in each tube is sufficient to perform 24 tests.	1x	4x
<b>ERY SPOT® INFORMATION CD</b>	With each kit there is a CD containing the Instructions for use for ERY SPOT® SSO Kits, for HISTO MATCH ERY SPOT® SSO Module and the user manual for MR.SPOT®. In addition the CD includes the batch file that has to be stored within the database of the HISTO MATCH ERY SPOT® Module interpretation software (for details see Instructions for use for HISTO MATCH ERY SPOT® Module).	1x	1x

\* In one kit there might be test wells from different sublots.

\*\* The mastermix can be used with all ERY SPOT® typing kits and is lot independent. The mastermix is sent separately with ice packs.

By ordering a kit with 96 Tests a mastermix **REF** 728500 (4 x 125 µl) will be delivered while by ordering a kit with 24 Tests a mastermix **REF** 728501 (1 x 125 µl) will be delivered.

\*\*\* XXX= COM (for ERY SPOT® Common)

RAR (for ERY SPOT® Rare)

RWD (for ERY SPOT® Weak D-TYPE)

For each kit there are lots and batches:

- **Kit:** e.g. ERY SPOT®
- **Lot:** defines the layout and specificity of the probes that are contained in the kit. A single lot can contain many different batches (sublots).
- **Batch (Sublot):** defines how a probe reacts in comparison to the control probes (cut off values), and defines the manufacture and expiry date of the strips.

### 3.2 Content of the HISTO SPOT® Reagent Kit

The reagents contained in one kit are sufficient for 96 tests. Please see table 2 for the detailed content of the HISTO SPOT® Reagent Kit.

**Table 2**

Component	Description
<b>BLOCKBUF</b>	<b>Blocking Buffer</b> , ready to use. It contains 0.001% Proclin® 150
<b>HYBBUF</b>	<b>Hybridisation Buffer</b> , ready to use. It contains 0.001% dye, 0.1% sodium dodecyl sulphate, 0.001% Proclin® 150
<b>STRGWASH</b>	<b>Stringent Wash Buffer</b> , ready to use. It contains 0.001% dye, 0.1% sodium dodecyl sulphate, 0.001% Proclin® 150
<b>TBSWASH</b>	<b>TBS Wash Buffer</b> (Tris Buffered Saline), ready to use. It contains 20 mM Tris, 0.003% dyes, 0.001% Proclin® 150
<b>SUBS</b>	<b>BCIP® / NBT Substrate</b> , ready to use. (5-bromo-4-chloro-3-indolyl phosphate / nitroblue tetrazolium chloride)
<b>CONJ</b>	<b>Conjugate</b> , Streptavidin Alkaline Phosphatase, concentrate. It contains < 0.1% sodium azide (to be diluted 1:1666 in Blocking Buffer)

### 3.3 Reagents and equipment required but not provided

- MR.SPOT® processor, including HISTO MATCH software, [REF](#) 726100
- Pipette tips for the MR.SPOT® processor, 1000 µl [REF](#) 726099 and 200 µl [REF](#) 726097
- DNA extraction reagents (no salting out method)
- Skirted PCR plates with lids or adhesive film (HISTO SPOT® PCR Frameplates, [REF](#) 726220, HISTO SPOT® PCR Caps, [REF](#) 726090, HISTO SPOT® PCR Foils, [REF](#) 726089)
- Deionized water
- Variable pipettes (range 0,5 – 1000 µl) and disposable tips
- Thermal cycler

## 4. STORAGE AND STABILITY

Testwells, primermix and primer solvent will be shipped at room temperature and have to be stored at 2...8 °C after delivery. The mastermix will be shipped with ice packs and has to be stored at ≤ -20 °C after delivery. The expiry date is indicated on the label of each reagent and is valid for the originally sealed reagents. The expiry date indicated on the outer box label refers to the reagent with the shortest shelf life contained in the kit. The mastermix can be used with all ERY SPOT® typing kits and is lot independent. The mastermix can have a shorter shelf life.

Individual strips of 8 wells may be opened and the required number of wells for a test run can be snapped off. The unused wells returned to the opened foil bag and stored for future use with the kit. Test wells stored in open foil pouches must be used within 30 days of being opened. The resuspended primermix solution should be stored at 2...8 °C and used within 30 days. The other opened reagents must be used within 3 months. The conjugate dilution must always be prepared afresh for each test run.

## 5. TEST PROCEDURE

### 5.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive methods and should be performed by well trained personnel, experienced in molecular genetic techniques. The results from these tests must not be used as the sole determinant for taking clinical decisions.

The current transfusion medicine guidelines as well as transfusion anamnesis should be followed in order to minimize the risk of false typing, especially in case of discrepancies between serological and molecular genetic methods. If no clear result can be obtained with the ERY SPOT® kits (e.g.

unknown alleles which cannot be determined with the existing primers or probes), the current transfusion guidelines must be applied to the serological results and the genotype should be determined by sequencing.

The test results should be evaluated taking into account the genetic variance of different ethnic groups. In case of doubts, refer to the serological determined phenotype.

Special safety conditions must be noted in order to avoid contamination and thus false reactions:

- ◆ Wear gloves during work (powder-free, if possible).
- ◆ Use new tips with each pipetting step (with integrated filter).
- ◆ Use separate working areas for pre-amplification (DNA isolation and preparation of the reactions) and post-amplification (hybridization and detection). Preferably use two separate rooms.
- ◆ Amplicon should not be taken back into PCR set up area.
- ◆ Use devices and other materials only at the respective places and do not exchange them.

## 5.2 DNA isolation

The use of a CE IVD certified extraction kit for DNA isolation is strongly recommend. Laboratory established standard method for DNA isolation should be validated by the user. Avoid the use of salting out methods, whether possible.

### Validate DNA extraction method:

- QIAGEN QIAamp DNA Blood Mini und Maxi Kit

The sample material for the isolation of genomic DNA should be sent in appropriate blood collection systems. The presence of heparin potentially inhibits PCR; therefore blood collection systems with heparin are not suitable. EDTA or Citrate blood is recommended for typing. A DNA concentration of 15-30 ng/μl is required to perform the test (see paragraph 5.3.3).

The purity indexes should be the following:

- extinction ratio  $OD_{260}/OD_{280}$ : > 1.5 and < 2.0  
Higher values indicate the existence of RNA, lower values mean contamination with protein.
- extinction ratio  $OD_{260}/OD_{230}$ : > 1.8  
Lower values indicate a possible contamination with carbohydrates, salts or organic solvents.

## 5.3 Amplification

### 5.3.1 Primer Solution preparation

Add 120 μl of primer solvent to each tube of dried primermix to resuspend the primermix and mix thoroughly (e.g. by vortexing). Allow the solution to stand for 15 minutes and then mix again until the primermix is completely resuspended.

#### Note:

- The dried primers should be completely resuspended before starting the test.
- The resuspended primer solution must be stored at 2...8 °C and used within 30 days.

### 5.3.2 PCR preparation

Use skirted PCR plates for the amplification, because they have to be held down at the skirt by a clamp in the MR.SPOT<sup>®</sup> processor afterwards. HISTO SPOT<sup>®</sup> PCR Frameplates have been validated for this application, PCR plates from other suppliers have to be validated by the user.

Thaw the mastermix. During pipetting store the mastermix in ice or inside an ice block. The mastermix should be mixed by pipetting before use. Do not vortex.

### 5.3.3 Performance of the PCR

For each sample to be amplified add the following reagents to each PCR tube:

- 4 µl Mastermix
- 11 µl Primer solution
- 5 µl Sample DNA (15-30 ng/µl)

Total volume for each amplification reaction is 20 µl.

**Note:** It is important that the total amount of DNA per test is in the range of 75 und 150 ng. Higher or lower DNA concentrations may result in false positive or false negative probe reactions. In addition lower concentrations may cause amplification failures. If frozen sample DNA should be used, avoid several freeze / thaw cycles.

If a negative control should be done, prepare one PCR reaction with distilled water instead of sample DNA.

Seal the amplification tubes with lids or adhesive film and shortly spin down the liquid.

Place in the thermal cycler and amplify under the conditions described in Table 3.

**Table 3**

Programme-Step	Time	Temperature	No. of Cycles
First Denaturation	2 Min	96°C	1 Cycle
Denaturation	15 Sec	96°C	10 Cycles
Annealing + Extension	1 Min	65°C	
Denaturation	10 Sec	96°C	20 Cycles
Annealing	50 Sec	61°C	
Extension	30 Sec	72°C	
Hold	∞	22°C	

The conditions are the same for all thermal cyclers, however the overall time required for this step will vary according to the ramping speed of the specific thermal cycler.

The following thermal cycler models haven been validated with ERY SPOT<sup>®</sup> SSO:

Eppendorf: Mastercycler<sup>®</sup> EP Gradient S (use the setting “simulate Mastercycler Gradient”)

Sensoquest Labcycler Gradient (Sensoquest) (use 2°C/sec ramp rate)

Biometra T professional 96

Applied Biosystems Veriti<sup>™</sup> Thermal Cycler (use the setting “9600 Mode”)

The use of a gold or a silver heating block is recommended.

If other thermal cyclers and/or other heating block are used, the validation has to be carried out by the user since false results cannot be excluded. It is generally recommended to use a ramp rate of 1-2°C/sec.

Once the amplification step is complete, the samples may be tested immediately or stored sealed at 2...8°C for up to 3 days.

It is not necessary to perform a gel electrophoresis as a control for the amplification. **There is no direct correlation between the intensity of the gel bands and the results of the ERY SPOT<sup>®</sup> assay. A weak gel band might correspond to a clear result.**

## 5.4 Automated hybridisation assay on the MR.SPOT<sup>®</sup> processor

### 5.4.1 Reagent preparation

Take HISTO SPOT<sup>®</sup> reagents and ERY SPOT<sup>®</sup> testwells out of the fridge and allow them to warm to room temperature.

Salt crystals may be observed in the hybridisation buffer and in the stringent wash buffer. If crystals are present, warm reagents up to 30°C to dissolve. Warm the whole content of the bottle, not an aliquot.

The conjugate has to be diluted 1:1666 in blocking buffer. The conjugate dilution must always be prepared afresh for each test run.

**The conjugate has to be vortexed and spun down each time before the dilution step!**

The required volumes of the reagents will vary depending on the number of strips to be tested. MR.SPOT<sup>®</sup> displays the required volumes for the chosen number of strips. Fill the required volumes of the reagents into the corresponding labelled reservoirs.

Place the test wells and the PCR plate into the appropriate blocks of the MR.SPOT<sup>®</sup> processor.

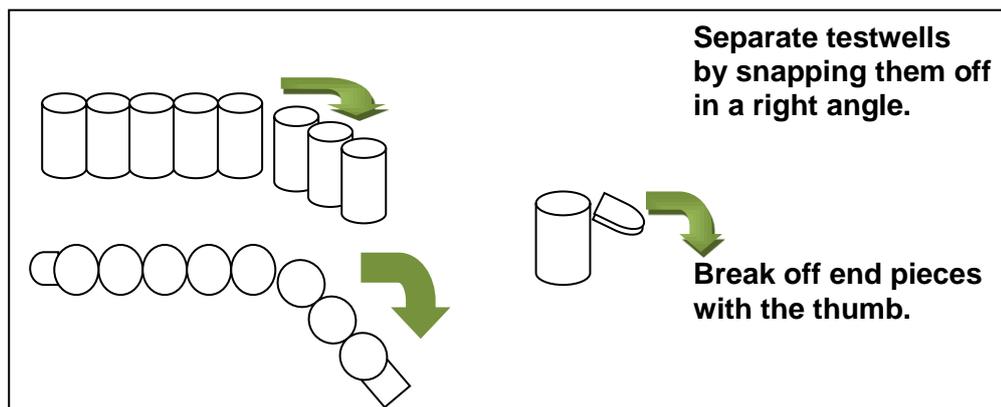
#### ATTENTION:

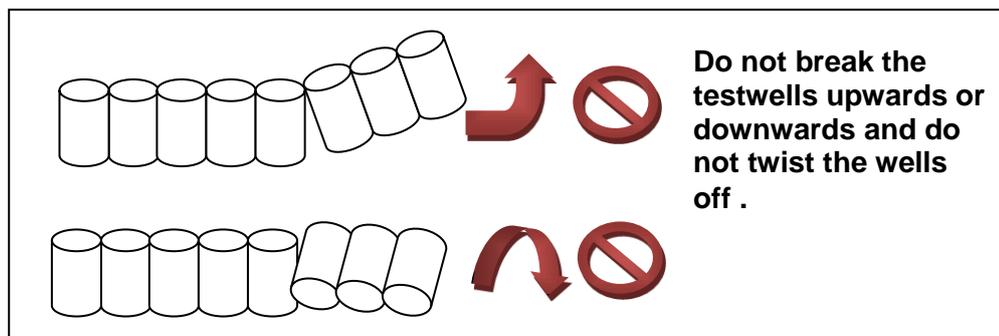
- Please make sure that orientation of the PCR Plate corresponds to the orientation of the testwells!
- Please make sure that the colour on the external bottom of the testwells corresponds to the colour displayed on the MR.SPOT<sup>®</sup> Processor's screen

Please make sure that there is no dirt or plastic particles in the reaction plate holder, because this may disturb the heat transfer during hybridization.

The teststrips can be separated in single wells according to figure 1, if less than eight tests should be run. If you are using separated wells be careful that they are sitting properly in the reaction plate holder and are not twisted against each other. You have to add clear dummy wells to reach multiples of four for the number of wells.

**Fig. 1: Separating single testwells.**





#### 5.4.2 Setup of the MR.SPOT<sup>®</sup> processor, transfer and interpretation of the results

Create a worklist in the HISTO MATCH ERY SPOT<sup>®</sup> SSO Module and send it to MR.SPOT<sup>®</sup> for processing. The worklist can be sent across a local area network directly to a MR.SPOT<sup>®</sup> processor or can be exported by using a USB memory stick.

Switch on the MR.SPOT<sup>®</sup> processor. The start up screen will appear. Select the previously created worklist and follow the process as indicated on the screen.

After a worklist has been processed on the MR.SPOT<sup>®</sup> processor, it needs to be imported along with the well images in the HISTO MATCH ERY SPOT<sup>®</sup> SSO Module. This can be done either via a local area network or with a USB memory stick.

The detailed processes are described in the User Manual for the MR.SPOT<sup>®</sup> processor and in the Instruction for Use for the HISTO MATCH ERY SPOT<sup>®</sup> SSO Module.

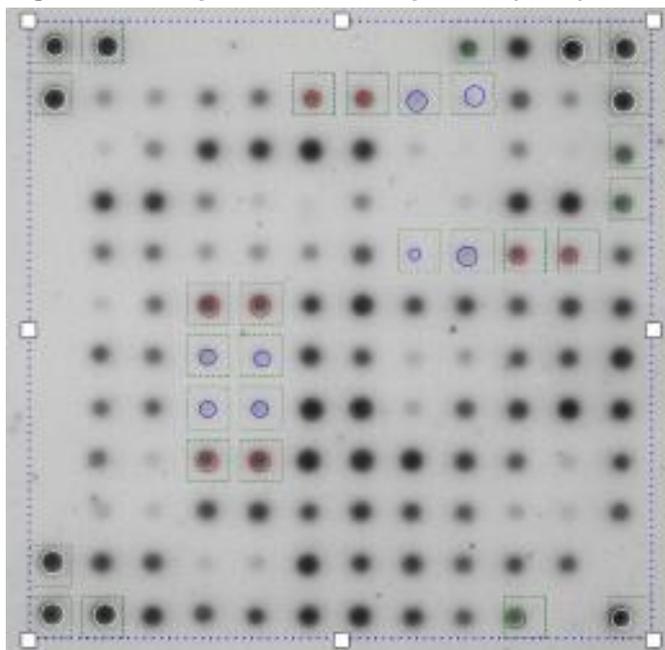
**Note:** The MR.SPOT<sup>®</sup> processor and the reagents should not be exposed to direct sun light.

#### 5.4.3 Interpretation of results

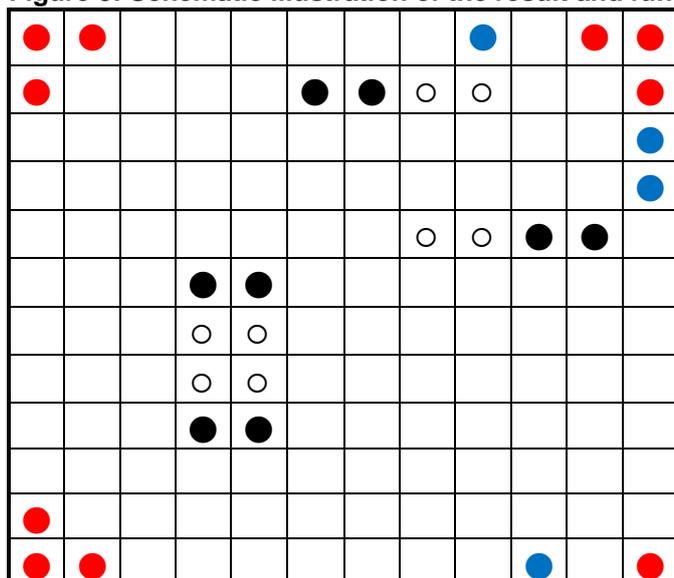
Open the HISTO MATCH ERY SPOT<sup>®</sup> SSO Module software and interpret the data as described in the Instructions for use for the HISTO MATCH ERY SPOT<sup>®</sup> SSO Module. The installation CD is delivered with the MR.SPOT<sup>®</sup> processor.

An example of the reaction pattern for ERY SPOT<sup>®</sup> Common is shown in figures 2 and 3. The reaction patterns of the other ERY SPOT<sup>®</sup> kits can differ from the one shown below. Figure 3 gives a schematic illustration of the result and the functions of the different probes.

**Figure 2: Example of a reaction pattern (MNS) for ERY SPOT<sup>®</sup> Common**



The colour of the circles around the probes indicate their function (see Instructions for use for the HISTO MATCH ERY SPOT<sup>®</sup> SSO Module for details).

**Figure 3: Schematic illustration of the result and function of the probes**

●: Positional probes. They are reacting with the amplification primers and indicate that during the SSO assay the PCR products were correctly transferred and that all reagents were added. Furthermore, they allow the software to locate the image (gridding).

●: Amplification control in duplicate. Those probes are universal for all alleles of the respective locus and show that the PCR was successful. They are also functioning as a reference for the allele specific probes.

●: Positive allele specific probe in duplicate

○: Negative allele specific probe in duplicate

## 6. WARNINGS AND PRECAUTIONS

ERY SPOT<sup>®</sup> SSO is designed for in vitro diagnostic use and should be used by properly trained, qualified staff. All work should be performed using Good Laboratory Practices.

Biological material used for extraction of DNA, e.g. blood or other body fluids, should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol.

Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

Blocking Buffer, Hybridisation Buffer, Stringent Wash Buffer and TBS Wash Buffer contain ProClin<sup>®</sup> 150. The reagents contain 0.001% preservative only, nevertheless avoid contact with the skin and mucous membranes.

The conjugate contains the preservative sodium azide. The reagent contains < 0.1% sodium azide which is not considered to be a harmful concentration. Nevertheless avoid contact with the skin and mucous membranes. Sodium azide may react with lead and copper plumbing to form explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide build-up.

All work with reagents should be handled with the appropriate precautions. Wear eye protection, laboratory coats and disposable gloves when handling the reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated.

If spills of reagents occur, dilute with water before wiping dry. Do not expose substrate to metals, oxidising agents.

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipettes and pipette tips is recommended. Do not use reagents with evidence of turbidity or microbial contamination.

## 7. SPECIFIC PERFORMANCE CHARACTERISTICS

### 7.1 Evaluation

Evaluation studies with pre-typed DNA samples have been performed for ERY SPOT® Common, Rare and Weak D-TYPE kits. The results were compared to results with other CE certified typing reagents (e.g. SSP, serology) and to sequencing.

For products certified according to the IVDD Annex II List A (ERY SPOT® Common and ERY SPOT® Weak D-TYPE) external and internal performance evaluation studies have been conducted by qualified personnel in various blood donor centers and medical laboratories, and in the BAG Health Care facilities, respectively.

#### 7.1.1 ERY SPOT® Common

Altogether 623 unselected samples were tested and analyzed for the KEL\*01.01 allele. The typing results showed 100% concordance with the SSP molecular genetic pretyping and concordance of at least 98.0% with the serological results.

See Tables 4 and 5 for details.

**Table 4**

Kell Serology			KEL*01.01 SSO			KEL*01.01 SSP		
Amount of tested samples	Amount of positive specificities	Amount of negative specificities	Amount of tested samples	Amount of positive alleles	Amount of negative alleles	Amount of tested samples	Amount of positive alleles	Amount of negative alleles
328	49 <sup>#</sup>	277 <sup>#</sup>	328	48	280	328	48	280
89	5	84	89	5	84			
86	22	64	86	22	64			
			120	3	117	120	3	117

# divergent results to SSO / Two samples show ambiguous serological results and therefore were excluded from the evaluation.

**Table 5**

Concordance (%)			
SSP with SSO		Serology with SSO	
Positive alleles	Negative alleles	Positive specificities	Negative specificities
100%	100%	98,0%	98,9%

The other blood group systems (Kidd, Duffy und MNS) of the ERY SPOT® Common kit showed 100% concordance with the molecular pretyping results.

#### 7.1.2 ERY SPOT® Rare

All tested blood group systems of the ERY SPOT® Rare kit showed 100% concordance with the molecular pretyping results.

### 7.1.3 ERY SPOT® Weak D-TYPE

1078 DNA samples were tested with the ERY SPOT® Weak D-TYPE kit, thereof 847 samples were serologically tested for the Rhesus D antigen. The results are shown in table 6. The typing results showed 96,7% concordance with the serological results.

**Table 6**

	Amount of tested samples	BAG	Total Extern 1-3
Total amount of tested samples (n)	1078	618	460
Thereof serologically tested (n)	847	477	370
Non concordant results between SSO and serology (n)	28	-	28
Concordance with serology (%)	96,7%		

311 DNA samples from the total amount of tested samples (n=1078) were molecularly typed to establish the presence of weak D alleles while 226 were molecularly tested for RHD\*01. The results are shown in table 7. The typing results showed 98,9% concordance with the molecular genotyping.

**Table 7**

		Amount of tested samples	BAG	Total Extern 1-3
Total amount of tested samples (n)		1078	618	460
Thereof molecularly tested (n)	RHD*01	226	226	-
	Weak D	311	230	81
Non concordant results between SSO and molecular genotyping (n)		6	1	5
Concordance with molecular genotyping (%)		98,9%	\	\

## 8. LIMITATIONS OF THE METHOD

The current transfusion medicine guidelines as well as transfusion anamnesis should be followed in order to minimize the risk of false typing, especially in case of discrepancies between serological and molecular genetic methods. If no clear result can be obtained with the ERY SPOT® kits (e.g. unknown alleles which cannot be determined with the existing primers or probes), the current transfusion guidelines must be applied to the serological results and the genotype should be determined by sequencing.

The test results should be evaluated taking into account the genetic variance of different ethnic groups. In case of doubts, refer to the serological determined phenotype.

References to undetectable alleles or to the presence of expression variants, e.g. RHDIIIa-CE (3-7)-D / DAU type 3, type 7) / partial-D (RHD\*DAU type 3) can be found in the software. This information is documented in the report.

Because of the high susceptibility of the PCR method to variations in DNA concentration and quality, only DNA samples should be used that have a concentration between 15 and 30 ng/μl and appropriate purity indices (extinction ratio  $OD_{260}/OD_{280}$ : between 1.5 and 2.0 / extinction ratio  $OD_{260}/OD_{230}$ : > 1.8).

Extreme care should be taken to prevent contamination of the kit reagents and other laboratory materials and equipment with amplicons or genomic DNA. Regular wipe tests to determine the presence of genomic DNA (e.g. Wipetest, [REF] 7091) and negative controls with each assay are recommended.

The hybridisation assay is a very temperature-sensitive process. Therefore, the ERY SPOT<sup>®</sup> SSO kits must be used in combination with the MR.SPOT<sup>®</sup> processor to ensure correct temperatures and incubation times.

All instruments (e.g. pipettes, thermal cyclers, heat blocks; MR.SPOT<sup>®</sup> processor) must be calibrated according to the manufacturers instructions. Accuracy and temperature uniformity of thermal cyclers may be tested with the CYCLER CHECK ([REF] 7104, 71044).

## 9. INTERNAL QUALITY CONTROL

Internal quality control of new lots of the ERY SPOT<sup>®</sup> SSO kits can be performed using a combination of DNA samples with known blood group type.

Internal positive controls are contained in each test well to ensure successful amplification and hybridization.

Negative controls to detect possible contaminations are recommended. Use a PCR reaction without DNA in the subsequent hybridization assay as a negative control.

## 10. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

Proclin<sup>®</sup> is a trademark of Rohm and Haas company.

BCIP<sup>®</sup> is a trademark of Sigma Aldrich Co.

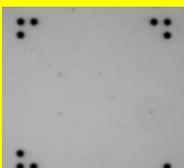
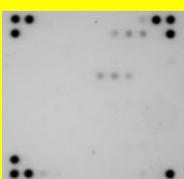
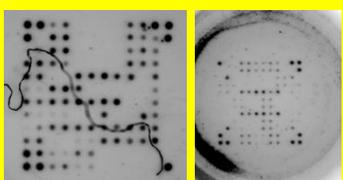
Mastercycler<sup>®</sup> is a trademark of Eppendorf AG.

Veriti<sup>™</sup> is a trademark of Applied Biosystems.

## 11. LITERATURE

- Daniels, G., *HUMAN BLOOD GROUPS*, Oxford, WILEY-BLACKWELL, 2013, ISBN: 978-1-4443-3324-4
- [www.rhesusbase.info/RHDDHMi.htm](http://www.rhesusbase.info/RHDDHMi.htm)
- <http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology>

**12. TROUBLESHOOTING**

Symptom	Possible problem(s)	Potential Solution(s)
Instrument Malfunction	Numerous	Refer to MR.SPOT <sup>®</sup> manual
Error message at data transfer	Failure in data transfer	Manually transfer data using USB drive
No result	Failure to grid image	Perform manual gridding
	Incorrect conjugate dilution	Repeat the test
	Almost or completely absent amplification due to sub-optimal DNA concentration or fragmented DNA	Check the DNA concentration
	Malfunctioning of MR.SPOT <sup>®</sup> Processor	Check the hybridization temperature on the instrument
Absence of spots in the well 	Failure to add <b>primermix</b> to PCR	Repeat the test
Only positional spots positive 	Failure to add DNA or <b>mastermix</b> to PCR or amplification failure	Repeat the test
False positive probes	Too much DNA used or conjugate concentration too high (not spun down).	Check DNA concentration. Spin down conjugate before use
No result / inconclusive result due to weak signals 	Incorrect conjugate dilution	Repeat the test
	Almost or completely absent amplification due to sub-optimal DNA concentration or fragmented DNA	Check the DNA concentration
	Incorrect mastermix volume	Repeat the test
	Malfunctioning of MR.SPOT <sup>®</sup> Processor	Check hybridization temperature on the instrument
No result / inconclusive result due to dirt in the well 	Precipitate or thread in the array	Repeat the test

**13. EXPLANATION OF SYMBOLS USED ON LABELING**

Symbol	Description
	For in vitro diagnostic use
	Storage temperature / Temperature limitation
	Storage temperature / Lower limit of temperature
	Manufacturer
	Electronic Instructions for use
	Batch code
	Use by
	Catalogue number
	Consult instructions for use
	Contains sufficient for <n> tests
	Intended purpose: blood group typing
	Mastermix for amplification of blood group alleles with ERY SPOT® SSO kits
	Primermix for amplification of blood group alleles with ERY SPOT® Common kits
	Primermix for amplification of blood group alleles with ERY SPOT® Rare kits
	Primermix for amplification of blood group alleles with ERY SPOT® Weak D-TYPE kits
	Primer Solvent for the ERY SPOT® Common kits
	Primer Solvent for the ERY SPOT® Rare kits
	Primer Solvent for the ERY SPOT® Weak D-TYPE kits
	Testwells with bound probes to type the blood group alleles with ERY SPOT® Common kits
	Testwells with bound probes to type the blood group alleles with ERY SPOT® Rare kits
	Testwells with bound probes to type the blood group alleles with ERY SPOT® Weak D-TYPE kits
	Blocking Buffer
	Hybridisation Buffer
	Stringent Wash Buffer
	TBS Wash Buffer (Tris buffered saline)
	BCIP® / NBT Substrate
	Conjugate, Streptavidin Alkaline Phosphatase
	CD, includes Instructions for use for ERY SPOT® SSO Kits, Instructions for use for HISTO MATCH ERY SPOT® SSO Module, User manual for MR.SPOT® Processor, Batch file

**14. ANNEX 1**

Product	Blood groups alleles
ERY SPOT® Common REF 728510 REF 728511	GYPA*01 (MNS*1) GYPA*02 (MNS*2) GYPB*03 (MNS*3) GYPB*04 (MNS*4) KEL*01.01 KEL*02 JK*01 JK*02 FY*01 FY*02 FY*null FY*weak
ERY SPOT® Rare REF 728520 REF 728521	KEL*02.06 KEL*02.07 KEL*02.03 KEL*02.04 DI*01 DI*02 CO*01.01 CO*02 DO*01 DO*02 YT*01 YT*02 LU*01 LU*02 VEL*01 (Vel+)* VEL*-01 (Vel-)*
ERY SPOT® Weak D-TYPE REF 728540 REF 728541	RHD*01 (RHD pos) RHD*01N.01 (RHD neg) RHD*01W.1 (RHD*weak D type 1) RHD*01W.1.1 (RHD*weak D type 1.1) RHD*01W.2 (RHD*weak D type 2) RHD*01W.3 (RHD*weak D type 3) RHD*09.03.01,09.04 (RHD*DAR3.01,DAR4 ) RHD*09.01.00 (RHD*DAR1.00) RHD*09.02.01 (RHD*DAR 2.01) RHD*01W.5 (RHD*weak D type 5) RHD*11 (RHD*weak partial 11) RHD*15 (RHD*weak partial 15) RHD*01W.17 (RHD*weak D type 17) RHD*01W.20 (RHD*weak D type 20) RHD*08N.01 (RHD*Pseudogene)

\* Replace the old nomenclature: Vel/SMIM1 reference (Vel+); Vel/SMIM1 64-80del (Vel-)

Instructions for use in other languages see:

<http://www.bag-healthcare.com>

or phone: +49 (0)6404-925-125