EN

Instructions for use

Wipe test

Contamination Control

Test kit for the detection of contaminations
on a molecular genetic basis

REF 7091

40 Reactions

1. Product description

To prevent contaminations in HLA typing [1] and for quality assurance in the laboratory working materials, laboratory areas or single reagents (e.g. Taq-Polymerase) should be monitored regularly for genomic DNA or amplificates (according to current EFI Standard).

The **Wipe test** is very well suited for the detection of contaminations with genomic DNA or amplificates of the HLA class I and II genes. The test procedure is based on the Sequence Specific Primers (SSP)-PCR (see Fig. 1) [2, 3]. This method makes use of the fact that primer extension, and hence successful PCR relies on an exact match at the 3'-end of both primers. Therefore, only if the primers entirely match the target sequence an amplification is obtained which is subsequently visualized by agarose gel electrophoresis.

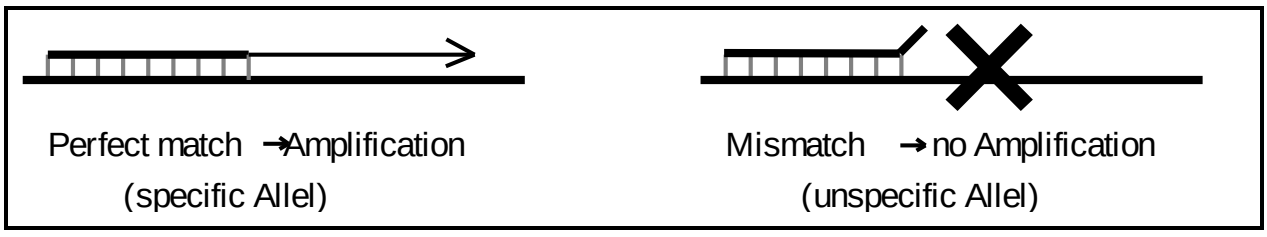

Fig. 1: Principle of SSP-PCR

Version: 7/2017 / Issued: 2017-04 **Changes to version 6/2016 are highlighted in yellow!**

2. Material

2.1 Contents of the Wipe test

- 5 PCR strips (à 8 PCR tubes) sufficient for 40 reactions (13 wipe tests). The prealiqoted and dried reaction mixtures consist of an allele specific primer set, internal control primers (specific for the human G3PDH gen) and nucleotides.
- 1 x 1.1 ml 10 x PCR-buffer
- 5 x strip-caps (à 8)
- Instructions for use

2.2 Supplementary material

- Taq Polymerase (5 U/µl), (Happy Taq (REF 70976) or another Taq Polymerase, validated with the Wipe test by the user)
  Don't use a Hot-start Taq Polymerase please!
- **BAG EXTRA-GENE I** Kit (REF 7059) (optional) for DNA extraction from blood/lymphocytes/ leucocytes or material for other DNA extraction methods
- piston pipettes (0.5-250 µl)
- sterile tips with integrated filter
- fleece paper
- DNA Cycler (validated cyclers please see page 4)

Devices and material for gel electrophoresis

- DNA agarose
- 0.5 x TBE buffer (45 mM of Tris, 45 mM of boric acid, 0.5 mM of EDTA)
- Ethidium bromide (EtBr)
- submarine electrophoresis unit with combs
- power supply (200 - 300 V, 200 mA)
- DNA-length standard (REF 7097)

Devices for interpretation and documentation

- UV source (220 - 310 nm)
- camera (e.g. Polaroid system) with films (Polaroid type 667) or video system with thermal paper (e.g. Typ KP65HM-CE)

2.3. Storage and stability

The kit is delivered at ambient temperature. Upon receipt please store the PCR strips at **≤ -20°C** or **2…8°C** in the dark (**Avoid frequent changes of the storage temperature!**). Store the 10x PCR buffer at **≤ -20°C**. Store in temperature monitored devices.
The expiry date is indicated on the label of each reagent and is also valid for opened reagents. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit.

3. Test procedure

3.1 Safety conditions

The PCR is a particularly sensitive method. Special safety measures must be observed in order to avoid contaminations 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 (gel electrophoresis, documentation). Preferably use two separate rooms.
- ♦ Use devices and other materials only at the respective places and do not exchange them.

3.2 DNA isolation

For the positive control DNA of leucocytes is required. The **BAG EXTRA-GENE I** kit is most suitable for the DNA isolation since pure DNA can be obtained from whole blood in a short time without the use of toxic chemicals or solvents. Furthermore commercial column- or beads-based methods or other methods described in literature are suitable to provide DNA of sufficient purity. The presence of heparin potentially inhibits PCR [6]. Therefore EDTA or Citrate Blood is recommended for typing.

DNA should have the following purity indexes:

- OD_{260}/OD_{280} = >1.5 and <2.0 (indicator for contamination with RNA/proteins)
- OD_{260}/OD_{230} = >1.8 (indicator for contamination with salt, carbohydrate or organic solvents

The evaluation and quality control of the Wipe test was done with DNA, which were extracted by EXTRA GENE I or Qiagen kits.

3.3 Amplification

The prealiquoted and dried reaction mixture already contains an allele specific primer set, internal control primers and nucleotides. Amplification parameters are optimized to a final volume of 20 µl. For each test three reactions are used.

The evaluation and quality control of the Wipe test was done with the Happy Taq (REF 70976).

3.3.1 Test procedure for the wipe test

1. 1.5 ml reaction vessels are labeled with the name of the examined areas (e.g workbench, door knob, ...) and filled with **200 µl sterile aqua dest**.
2. For each test area a piece of fleece is dipped into the respective reaction vessel and the test area is wiped with the wet fleece.
3. Put the fleece in the respective reaction vessel and incubate for 2 h at room temperature in the 200 µl of aqua dest. After this time the fleece is discarded.

4. Remove the required number of PCR vessels from the kit and thaw the 10x PCR-buffer. Label one vessel with “test area”, one with “positive control” and the third one with “inhibition control”.

5. Prepare the **Taq-predilution** (minimum 5 reactions) and briefly vortex the mixture.

Preparation of Taq-predilution for number of reactions + 2 :

	pro 1 reaction	**5 reactions**	**8 reactions**
10x PCR-buffer	2 µl	10.0 µl	16 µl
Taq Polymerase (5 U/µl)	0.12 µl	0.60 µl	0.96 µl

6. Pipet the following reaction mixes in the labeled PCR vessels:

	test area	**positive control**	**inhibition control**
sterile aqua dest	14 µl	17 µl	13 µl
sample of test area	4 µl	-	4 µl
genomic DNA (40ng/µl)	-	1 µl	1 µl
Taq-predilution	2 µl	2 µl	2 µl

7. Tightly close the tubes with the respective caps. Ensure that you do not touch the inner side of the caps and the upper edges of the tubes with the fingers to avoid contamination. Powder of gloves is a strong inhibitor of PCR! Slightly shake the strip to dissolve the pellet at the bottom of the strip. All PCR solution should be settled on the bottom. If necessary the strip should be briefly spun down.

8. Place the reaction tubes firmly into the thermal cycler and tighten lid well. Start the PCR programme. Overlaying of the reaction mixtures with mineral oil is **not** required if a heated and adjusted lid is used!

Amplification parameters

Programme-Step	**Temp.**	**Time**	**No. of Cycles**
First Denaturation	96°C	**5 Min**	1 Cycle
Denaturation	96°C	20 Sek	5 Cycles
Annealing+Extension	68°C	60 Sek	
Denaturation	96°C	20 Sek	10 Cycles
Annealing	64°C	50 Sek	
Extension	72°C	45 Sek	
Denaturation	96°C	20 Sek	15 Cycles
Annealing	61°C	50 Sek	
Extension	72°C	45 Sek	
Final Extension	72°C	5 Min	1 Cycle

Validated Cycler types:
PTC 100 / 200 / C1000 (MJ Research/ BioRad), GeneAmp PCR-System 9600 / 9700 (use heating rate of 9600 please), Veriti (ABI), Mastercycler epGradient S (use “simulate Mastercycler gradient” function please) (Eppendorf) and Tprofessional (Biometra)

Please don’t use an aluminium heating block (e.g. GeneAmp PCR-System 9600 / 9700). When using thermal cyclers with a very fast heating and cooling rate, it is recommended to use a reduced ramp rate (~2.5°C/sec).

Since cyclers of different manufacturers perform differently and even individual machines of one type may be calibrated differently, it may be necessary to optimize the amplification parameters. If other models than the validated cyclers mentioned above are used they have to be validated by the user.

To optimize your machine use the following guide:

If there are **false positive** reactions (unspecific bands), increase the annealing temperature in 1°C steps.

If there are **false negative** reactions (missing bands and/or amplification controls), decrease the annealing temperature in 1°C steps and/or increase the annealing times in 5 second steps and/or increase the denaturation times in 5 second steps.

It is recommended to use exclusively cyclers that are calibrated regulary. For this the CYCLER CHECK kit is well suited (REF 7104, 71044).

The quality control tests were done on a PTC-200 resp. C1000 (MJ Research / BioRad), 9700 (ABI), Mastercycler epGradient S (Eppendorf) and Tprofessional (Biometra).

3.4 Gel electrophoresis

Separation of the amplification products is done by electrophoresis via a (horizontal) agarose gel. As electrophoresis buffer, 0.5 x TBE (45 mM of tris, 45 mM of boric acid, 0.5 mM of EDTA) buffer is recommended. The gel concentration should be 2.0 - 2.5% of agarose. Allow the gel to polymerize at least 30 minutes before sample loading. After amplification has been finished, take the samples out of the thermal cycler and load the complete reaction mixtures carefully in each slot of the gel. In addition, apply 10 µl of the DNA length standard for size comparison. Electrophoretic separation is done at 10 - 12 V/cm (with 20 cm distance between the electrodes approx. 200 - 240 V), for 20 - 40 minutes. After the run has been completed, the complete gel is stained in an ethidiumbromide (EtBr) solution (approx. 0.5 µg/ml of EtBr in H_2O or TBE buffer) for 30 - 40 minutes. As alternative, EtBr (0.5 µg/ml) can also be added to the electrophoresis buffer or the agarose gel. If required, excess of EtBr can be removed by soaking the gel in H_2O or 0.5 x TBE buffer for 20 - 30 minutes.

3.5 Documentation and interpretation

For documentation, visualize the PCR amplification using an UV transilluminator (220 - 310 nm) and photograph it with a suitable camera, film and filters (e.g. Polaroid, film type 667 or video system, thermal paper KP65HM-CE). Choose exposure time and aperture such that the bands are drawn sharp and stand out against the dark background.

If the test area is not contaminated **no** band should be visible in the test area sample.

Contaminations are indicated by the following bands:

Contamination with amplificate:	**78 bp** and/or **104 bp** and/or **282 bp**
Contamination with genomic DNA:	**282 bp** and possibly **78 bp**, **104 bp**, **176 bp**, ca. **580 bp**

The positive control and the inhibition control should exhibit a band pattern according to the one expected with genomic DNA. If there are no amplificates in the positive control, no PCR reaction has taken place and the whole test cannot be interpreted. If the positive control shows the correct band pattern, but there are no bands visible in the inhibition control, inhibitors must have been present in the test area. In this case a clean “test area” sample is no proove that there are really no contaminations present in the test area.

4. Warnings and Precautions

Ethidiumbromide is a powerful mutagen. Wear gloves when handling gels or solutions containing EtBr! Note the instructions for use and warnings and precautions of the manufacturer! The transilluminator radiates very short-wave UV light which may cause burns of the skin and the retina. Use a UV face safety mask!

All for extraction of DNA used biological material, e.g. blood or human tissue, 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).

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

A declaration on Material Safety Data Sheets (MSDS) is available to download at www.bag-healthcare.com .

5. References

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2. Olerup, O., Zetterquist H., 1992. Tissue Antigens **39**:225-235
3. Olerup, O., Zetterquist H., 1993. Tissue Antigens **41**:55-56
4. Lu, Y.H. and Négre, S., 1993. Trends in Genetics **9**:297
5. Green and Sambrook, 2012. Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbour Laboratory
6. Beutler, E. et al., 1990. BioTechniques **9**:166
7. Bunce, M., 1995. Tissue Antigens **46**:355-367

6. Explanation of symbols used on Labelling

	Storage temperature
	Use by
	Consult instructions for use
	Caution
	Sufficient for n tests
CONT	Content, contains
CONTROL | CC	Contamination Control
IFU	Instructions for use
LOT	Batch code
OR	Or
PCRBUF | 10x	PCR buffer, 10x concentrated
PCRCAP	PCR caps
PCRSTRIP	PCR strips
REACTIONMIX	Reaction mixes
REF	Catalogue number
RTU	Ready to use

Instructions for use in other languages see:
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