Instructions for use

HISTO TYPE / DNA-Wipetest

Contamination Control

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

REF *7091*

40 Reactions

1. Product description

The use of Polymerase Chain Reaction (PCR①) in HLA typing has become routine in the recent years. Since the PCR is a very sensitive method is it essential to take precautions to avoid contaminations which would lead to false-positive reactions. To prevent contaminations and to secure the quality in a laboratory working materials, laboratory areas or single reagents (e.g. Taq-Polymerase) should be monitored regularly for DNA or amplificates.

The **HISTO TYPE / DNA-Wipetest** 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 (SSP②) -PCR① (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 visualised by agarose gel electrophoresis.

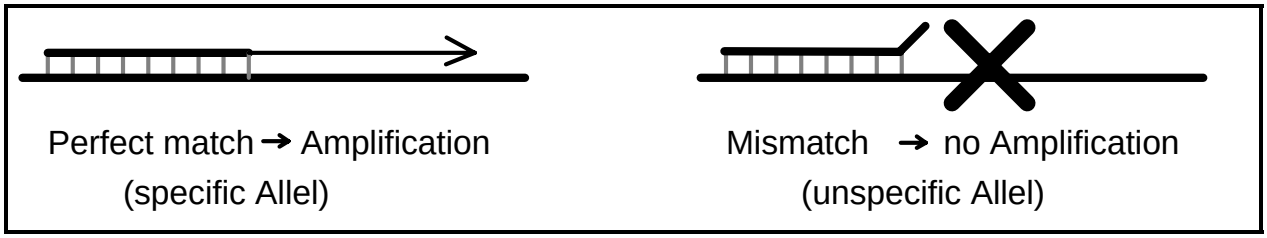

Fig. 1: Principle of SSP-PCR

2. Material

2.1 Contents of the HISTO TYPE / DNA-Wipetest

- 5 HISTO TYPE strips (à 8 thinwalled PCR tubes) sufficient for 40 reactions (13 wipetests). 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 8er strip-caps
- instructions for use

2.2 Supplementary material

- Taq Polymerase (5 U/µl)
- **BAG EXTRA-GENE** Kit 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 (e.g. PTC 200 with a heated and adjusted cover, MJ Research/Biozym)

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 (Cat.-No.: 7097)

Devices for interpretation and documentation

- UV source (220-310 nm)
- camera (e.g. Polaroid system) with films (Polaroid type 667)

2.3. Storage and stability

The kit is delivered unfrozen. Store all reagents at -20…-80°C in the dark. The expiry date is indicated on the label of each reagent and is also valid for opened reagents. Stability as indicated on the outer label refers to all reagents contained in the kit. Shortly before using, thaw the 10 x PCR-buffer.

3. Test procedure

3.1 Safety conditions

The PCR① 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. E.g. the **BAG EXTRA-GENE** kit is most suitable for isolation since pure DNA can be obtained from whole blood in a short time without the use of toxic chemicals or solvents. Other methods described in literature [5] such as the cetyltrimethyl-ammonium-bromide (CTAB) method or phenol-chloroform purification are as well suitable to provide DNA of sufficient purity. The presence of heparin potentially inhibits PCR① [6]. Therefore EDTA or Citrate Blood is recommended. DNA should have a purity index (extinction ratio OD_{260}/OD_{280}) between 1.6 and 2.0.

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.

3.3.1 Test procedure for the wipetest

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 HISTO TYPE vessels from - 20°C and thaw the 10 x PCR-buffer at room temperature. 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 so that contamination is avoided. Powder of gloves is a strong inhibitor of PCR! Slightly shake the plate downwards to dissolve the blue pellet at the bottom of the plate. All PCR solution should be settled on the bottom.

8. Place the reaction tubes into the thermocycler and tighten lid so that the reaction vessels do not warp in heating. Start the PCR① programme. Overlaying of the reaction mixtures with mineral oil is **not** required if a heated and adjusted cover is used!

Amplification parameters:

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

Cycler types:
PTC 100 / 200
(MJ Research/ Biozym)
and
GeneAmp PCR-System
9600 / 9700
(Perkin Elmer)

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.

To optimize your machine use the following guide:
With **false positive** reactions (unspecific bands, additional types): Increase of the annealing temperature in 1°C steps.

With **false negative** reactions (bands missing): Decrease of the annealing temperature in 1°C steps and/or increase of the annealing periods in 5 second steps and/or increase of the denaturation periods in 5 second steps.

It's recommended to use only regularly calibrated cyclers. For this the BAG-Cycler Check kit is very suitable (Cat.-No.: 7104).

The quality control tests were done on a PTC-200 resp. 100 (MJ Research) and 9700 (ABI).

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 thermocycler 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 min.. 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). 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). Choose exposure time and aperture such that the bands are drawn sharp and stand out against the dark background (approximates aperture 11, exposure time 1 second).

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 immediatley 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).

5. References

1. Bodmer, J., 1993. Immunogenetics **37**:79-94
2. Olerup, O., Zetterquist H., 1992. Tissue Antigens **39**:225-235
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4. Lu, Y.H. and Négre, S., 1993. Trends in Genetics **9**:297
5. Maniatis et al., 1989. Molecular Cloning: A Laboratory Manual.
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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
LOT	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use

① **Notice to Purchaser: Disclaimer of License**

This product is optimized for use in the Polymerase Chain Reaction ("PCR") Process which is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser by the purchase of this product. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California, 94501.

②The SSP-Method corresponds with the covered name ARMS™ of the firm ZENECA, Manchester. This method is covered under the european patent number 0 332 435 B1 and is used with the consent of the firm ZENECA.

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