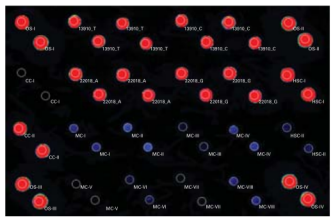
EUROArray Lactose Intolerance Direct



- **Parallel detection of the polymorphisms associated with primary lactose intolerance, 13910 C/T and 22018 G/A, in one test**
- **Direct use of EDTA blood: no separate DNA isolation required**
- **Highest reliability of results due to included controls for interfering neighbouring mutations**

Technical data

Substrate	Single-stranded DNA probes, length: 15 to 50 nucleotides
Test procedure	DNA extraction / PCR (approx. 60 min) / hybridisation (60 min) / fully automated evaluation Total working time approx. 2 min per sample incl. DNA extraction with the direct method (with 40 samples per run)
Reagents	Ready for use
Controls	DNA-negative control and other integrated controls
CE IVD label	Complete process incl. DNA extraction is validated
Test kit format	5, 10 or 20 slides, each containing 5 test fields, or 8 slides each containing 3 test fields
Order no.	**MN 5351-0505-V, -1005-V, -2005-V, -0803-V**

Clinical significance

The EUROArray Lactose Intolerance Direct provides molecular genetic determination of the two polymorphisms most frequently associated with primary lactose intolerance, 13910 C/T and 22018 G/A, which are localised in the promotor region of the lactase (LCT) gene. Primary lactose intolerance is based on a genetically caused deficiency of the digestive enzyme lactase in the intestine, which is responsible for breaking down the disaccharide lactose into its sugar monomers glucose and galactose. Unsplit lactose is fermented in the ileum and the large intestine, resulting in fermentation products which cause digestive disorders and the typical symptoms of lactose intolerance. These include abdominal pain, nausea, meteorism and diarrhoea. Secondary manifestations of the disease can include deficiencies (e.g. vitamin deficiency) and, as a result, unspecific symptoms such as fatigue, chronic tiredness and depression. Around 20 % of Europeans and almost 100 % of the normal population in large parts of Asia and in the south of Africa suffer from primary lactose intolerance. However, there are mutations which lead to a continuously increased lactase production and consequently to a tolerance to lactose (lactase persistence). According to the current state of knowledge, homozygous carriers of 13910 C/C and 22018 G/G develop symptoms of lactose intolerance, while heterozygous carriers of 13910 C/T- and 22018 G/A only present symptoms in situations of stress or with intestinal infections. Homozygous carriers of 13910 T/T and 22018 A/A are lactase-persistent and do not show any symptoms.

Since there is not only the genetically caused form of (primary) lactose intolerance (below 50% lactase activity), but also a secondary lactose interolance which can usually be overcome within some months, it is important for patients to clarify the exact cause of the disease. Alongside molecular genetic test systems, indirect serological detection methods such as hydrogen breath test (H_2 breath test), IgE or IgG antibody test, or blood sugar tests are performed. These, however, cannot distinguish between the primary and the secondary form of lactose intolerance due to their low specificity and extremely low sensitivity. Consequently, the genetic diagnostic detection is required for diagnostic clarification of lactose intolerance with reliable and exact diagnostic accuracy, alongside the assessment of clinical symptoms.

Diagnostic application

The EUROArray Lactose Intolerance Direct enables quick and simple determination of the two polymorphisms 13910 C/T and 22018 G/A in the regulatory region of the LCT gene in a single test. In the direct method full blood samples can be used directly without the need for DNA isolation, saving time and costs.

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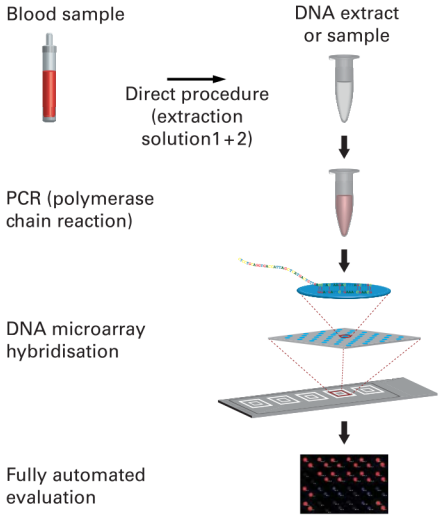
Test principle

This test kit provides molecular genetic in vitro determination of the two polymorphisms 13910 C/T (rs4988235) and 22018 G/A (rs182549) in the regulatory region of the LCT gene. EDTA blood (direct method) or isolated genomic DNA from the patient are used as sample material. In the direct method genomic DNA from blood cells is prepared for polymerase chain reaction (PCR) by diluting the blood with the extraction solution provided in the test kit and incubating it for one minute. In the first reaction step, several sections of the LCT gene are amplified by the polymerase chain reaction (PCR) from the extract or, alternatively, from a genomic patient DNA sample. The PCR products are labelled with a fluorescence dye as they are produced. In the second reaction step, the products are analysed using the microarray, which contains allele-specific immobilised probes in the form of small round spots that are complementary to the amplified DNA. The specific binding (hybridisation) of the fluorescing PCR product to the corresponding microarray spot is detected using the EUROArray Scanner (EUROIMMUN). All spot signals are automatically evaluated by the EUROArrayScan software and the genotype is deduced from the signals generated by the allele-specific probes.



Test performance

For direct use of EDTA blood, the sample is first incubated with extraction solution 1 for one minute and then extraction solution 2 is added. For PCR an aliquot of the extract or alternatively a purified DNA sample is mixed with the ready-made PCR reagents. The PCRs are incubated in the thermocycler and then, using the TITERPLANE technique, on EUROArray slides containing microarray BIOCHIPs. Scanning and evaluation are performed using the EUROArray Scanner (scanner, incl. EUROArrayScan software). This provides fully automated evaluation of EUROArray analyses and detailed documentation of results.

Sensitivity and specificity

Sensitivity and specificity of the test system were determined with samples precharacterised using a molecular genetic method.

Reference samples	Reference method	Sensitivity with resp. to reference method	Specificity with resp. to reference method
85 EDTA blood samples[1] from blood donors, Germany	molecular genetic	100 %	100 %

Robustness

For 203 analysed samples[1] from blood donors, the determination was successful in all cases (100 %).

Prevalence

In the investigation of 152 randomly selected samples from blood donors the following genotypes were determined:

Genotype		Prevalence
13910 C/T:	T/T (homozygous T)	31.6 %
	C/T (heterozygous)	48.0 %
	C/C (homozygous C)	20.4 %

Genotype		Prevalence
22018 G/A:	A/A (homozygous A)	32.2 %
	C/A (heterozygous)	48.0 %
	G/G (homozygot G)	19.7 %

[1]The analyses were performed both with EDTA blood (with the direct procedure), and DNA samples isolated from EDTA blood samples using the "QIAamp® DSP DNA Blood Mini Kits" (QIAGEN) according to the manufacturer's instructions.

Literature

1. Deng Y, Misselwitz B, Dai N, Fox M. Lactose Intolerance in Adults: Biological Mechanism and Dietary Management. Nutrients. 2015 Sep 18;7(9):8020-35.
2. Dzialanski Z, Barany M, Engfeldt P, Magnuson A, Olsson LA, Nilsson TK. Lactase persistence versus lactose intolerance: Is there an intermediate phenotype? Clin Biochem. 2016 Feb;49(3):248-52.

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