EN

Instructions for Use

FastQ CD

Test kit for determination of HLA-DQ on a molecular genetic basis

Electronic instructions for use see www.bag-diagnostics.com

IVD

REF 728202 FastQ CD

CE

Contents

Changes to version 1/2020 are marked in yellow.

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BAG Diagnostics GmbH
Amtsgerichtsstr. 1-5
35423 Lich/Germany
Tel.: +49 (0) 6404 / 925-100
Fax: +49 (0) 6404 / 925-460
www.bag-diagnostics.com
info@bag-diagnostics.com

Ordering:
Tel.: +49 (0) 6404 / 925-450
Fax: +49 (0) 6404 / 925-460
order@bag-diagnostics.com

Customer Service:
Tel.: +49 (0) 6404 / 925-125
Fax: +49 (0) 6404 / 925-421
service@bag-diagnostics.com

1. INTENDED USE

The intended use of the FastQ product line is human genetic testing for markers that are associated with diseases or pharmacogenetic reactions. For the **FastQ CD** kit this is the determination of the presence of HLA-DQ alleles that are associated with coeliac disease (see Product Description).

2. PRODUCT DESCRIPTION

The **FastQ CD** kit is used for the molecular genetic detection of HLA-DQ alleles that are associated with coeliac disease. Coeliac disease is an autoimmune disorder mainly affecting the small intestine and is caused by a reaction with gluten. Gluten is part of different grains. The exposure to gluten leads to abnormal immune response in genetically predisposed people causing inflammatory reactions and long term distorsion of the small intestine. Coeliac disease is strongly associated with the DQ-genotypes HLA-DQA1*02:01, HLA-DQA1*05:05, HLA-DQA1*05:01, HLA-DQB1*03:02, HLA-DQB1*02:01, HLA-DQB1*02:02. Genetically predisposed people have a up to 40-times higher risk to be affected by coeliac disease. Therefore, the detection of these haplotypes is used in diagnostics (1-3). Confirmed diagnostic results of HLA-DQ-haplotypes make an important contribution to the therapy of a patient (gluten-free food). The **FastQ CD** kit detects all DQ2/DQ8 haplotypes associated with coeliac disease.

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 specific parts of the HLA-DQ gene. The amplicons are detected with likewise gene locus specific fluorescent dye-labelled hydrolysis probes (TaqMan® probes), which increases the specificity of the test compared to a conventional SSP.
If amplicons are present, the probes are hydrolyzed by the Taq polymerase and a fluorescence signal is generated that increases proportionally to the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the RT-PCR-Cycler.
The test is performed with two PCR mixes that detect the internal positive control (*human growth hormon*) and the disease-associated haplotypes with different fluorescent colours.

4. MATERIAL

4.1 Contents of the FastQ CD kit (48 Tests)

- **130 µl Q Primermix CD I**, ready to use, contains primers and probes
- **130 µl Q Primermix CD II**, ready to use, contains primers and probes
- **260 µl Plex Mix**, ready to use, contains dNTPs,Taq Polymerase, reaction buffer
- **Instructions for use**

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Fax: +49 (0) 6404 / 925 - 460
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4.2 Additionally required reagents and devices

- Reagents for DNA isolation (validated DNA isolation kits see 6.2)
- Real-Time PCR-Cycler (validated cyclers see 4.3)
- RT-PCR reaction tubes with caps or foils (validated products see 4.3)
- Aqua dest. (DNAse free)
- Piston pipettes (0,5 – 1000 µl) and tips
- Centrifuge (e.g. PlateFuge – MicroCentrifuge by Benchmark Scientific)
- Colour Compensation kit for LightCycler® 480 II (provided by BAG Diagnostics)

4.3 Validated cyclers and reaction tubes

Cycler	RT-PCR reaction tubes	RT-PCR closing systems
CFX96™ Real-Time PCR Detection System Comp. Bio-Rad	FrameStar® Break-A-Way PCR Plate 96 white wells, black frame Product No. 4ti-1201 Comp. 4titude / Brooks Life Sciences	Crystal Strips Product No. 4ti-0755 Comp. 4titude/Brooks Life Sciences qPCR Seal (Optically clear adhesive film) Product No. 4ti-0560 Comp. 4titude/Brooks Life Sciences
LightCycler® 480 II Comp. Roche	Roche Multiwell Plate 96, white Product No. 04729692001 Comp. Roche	qPCR Seal (Optically clear adhesive film) Product No. 4ti-0560 Comp. 4titude/Brooks Life Sciences

Note: If other realtime cyclers, reaction tubes and closing systems are used they must be validated by the user.

5. STORAGE AND STABILITY

The kits are shipped on blue ice. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. 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 has shown that up to 6 cycles for the Plex Mix and up to 15 cycles for the Q Primermixes has no detrimental effects on the quality of the kit. No data are available yet for more cycles. Therefore, it is recommended to aliquot the reagents if necessary.

6. TEST PROCEDURE

6.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques. The results of these tests must not be used as sole basis for clinical decisions.

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Special safety conditions must be observed 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).
- If possible, use separate working areas for pre-work (DNA isolation and PCR set up) and post-amplification (detection and PCR).
- Use devices and other materials only at the respective places and do not exchange them.

6.2 DNA Isolation

The sample material for the isolation of genomic DNA must be sent in appropriate blood collection systems. For the test EDTA or Citrate blood is required. The presence of heparin potentially inhibits PCR; therefore blood collection systems with heparin are not suitable (4) and must not be used.

It is recommended to use CE IvD certified kits for the DNA isolation.

Validated DNA isolation kits:

- Qiagen QIAamp DNA Blood Kits (columns)

If the established standard method of the lab is used for gDNA isolation and this is not the validated kit above, it must be validated by the user.

A DNA concentration of 10 - 50 ng/µl is required to perform the **FastQ CD** test.

The DNA must have the following purity indexes:

- OD_{260}/OD_{280} = > 1.5 and < 2.0
  Higher values are an indicator for contamination with RNA, lower values for a contamination with proteins.
- OD_{260}/OD_{230} = > 1.8
  Lower values indicate a contamination with salt, carbohydrate or organic solvents.

6.3 Amplification

Reaction tubes recommended by the manufacturer of the real-time cycler or the materials recommended in chapter 4.3 should be used.

For each sample the following reagents are pipetted into a reaction tube:

Reaction I		Reaction II	
2 µl	Q Primermix CD I	**2 µl**	Q Primermix CD II
2 µl	Plex Mix	**2 µl**	Plex Mix
1 µl	Sample DNA (10-50 ng/µl)	**1 µl**	Sample DNA (10-50 ng/µl)
5 µl	Aqua dest. (DNAse free)	**5 µl**	Aqua dest. (DNAse free)

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The reaction volume for each RT-PCR test is 10 µl.

If a premix of Q Primermix, Plex Mix and Aqua dest. is prepared for more than one sample please allow for a reasonable additional amount for pipetting losses.

If a **negative control (NTC)** should be performed prepare a PCR reaction with Aqua dest. instead of DNA.

Close the reaction tubes and briefly spin down the liquid. Ensure that no bubbles are present in the wells. If bubbles are observed, gently tap assay on the bench to remove the bubbles. Start the PCR program with the following parameters:

Step	Time [s]	Temperature [°C]	Ramp rate [°C/s]	Plate read	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	

The following realtime cyclers have been tested for the **FastQ CD** kit:

Bio-Rad: CFX96™ Real-Time PCR Detection System

Roche: LightCycler® 480 II

Remark

When using the LightCycler® 480 II system a colour compensation is required (provided by BAG Diagnostics).

6.4 Interpretation of results

All tests with human gDNA must show a fluorescence signal in the green channel (FAM) with the internal control.

Amplification signals for the samples that are negative for the detectable DQ alleles should be outside the defined Cq values for the three channels. A negative control with Aqua dest. should not show any fluorescent signal during the complete RT-PCR run and represents a contamination control. Fluorescence signals within the defined Cq values with the negative control with Aqua dest. indicate contamination. Flourescence signals outside the defined Cq values can occur due to the very sensitive test method in case of inaccurate pipetting. If this occurs, the test should be repeated.

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Samples with the HLA-DQ alleles listed below show a positive signal in the following channels for the CFX-Cycler:

Q Primermix CD I	Fluorophore / Colour channel	Cq Level	Wavelength [nm]
DQB1*02:01	CAL Fluor Red 610 / Texas Red	< 20	Excitation: 590 / Emission: 610
DQA1*05:05	Quasar 670 / Cy5	< 20	Excitation: 647 / Emission: 670
DQA1*02:01	CAL Fluor Orange 560 / HEX	< 15	Excitation: 538 / Emission: 559
IAC	FAM	< 18	Excitation: 495 / Emission: 520

Q Primermix CD II	Fluorophore / Colour channel	Cq Level	Wavelength [nm]
DQB1*02:02	CAL Fluor Red 610 / Texas Red	< 16	Excitation: 590 / Emission: 610
DQA1*05:01	Quasar 670 / Cy5	< 16	Excitation: 647 / Emission: 670
DQB1*03:02	CAL Fluor Orange 560 / HEX	< 17	Excitation: 538 / Emission: 559
IAC	FAM	< 18	Excitation: 495 / Emission: 520

The Cq level defines the latest Cq number when a positive reaction (fluorescence rises above the threshold) is expected in the respective channel. The threshold that is automatically set by the CFX software should be used as baseline threshold.
It is recommended to check the plausibility of the reactions with the amplification curves and to repeat questionable results. If there are questions regarding the adaptation of the threshold or borderline Cq values please contact the technical support of BAG Diagnostics (phone: +49 (0)6404 925125, email: info@bag-diagnostics.com).

BAG Diagnostics GmbH
Amtsgerichtsstr. 1-5
35423 Lich/Germany
Tel.: +49 (0) 6404 / 925-100
Fax: +49 (0) 6404 / 925-460
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info@bag-diagnostics.com

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Fax: +49 (0) 6404 / 925-421
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6.5 Specificity of the kit

The following alleles are detected by the kit:

Primermix I	Common*	Well documented*	Rare*
CAL Fluor Red 610	**DQB1*02:01:01:01,** *02:03:01		DQB1*02:01:04-08, 15-37, *02:03:01/02, *02:07:01/02, *02:08, *02:09, *02:14:01/02, *02:27, *02:28?-*02:47? , *02:48, *02:49?-*02:52? , *02:53Q, *02:54?-*02:56? , *02:57, *02:48N? , *02:59, *02:60? , *02:61? , *02:63, *02:66?-*02:71?, *02:72, *02:73?-*02:78?, *02:79, *02:81-*02:83, *02:85?-*02:88? , *02:90?-*02:94? , *02:96N, *02:98, *02:99, *02:100? , *02:101? , *02:102, *02:103? , *02:104? , *02:105-*02:109, *02:111, *02:112, *02:114, *02:115, *02:118, *02:119, *02:123, *02:125, *02:128, *02:129N? , *02:130, *02:132N-*02:136, *02:139? , *02:140? , *02:148, *02:149, *02:151?, *02:152, *02:154, *02:155, *02:157-*02:160, *02:163N, *02:164, *02:166
Quasar 670	**DQA1*05:05:01:01**, *05:08	DQA1*05:02?, *05:09	DQA1*05:04?, *05:05:01:02-22, *05:05:02-04, *05:10?, *05:11, *05:12?, *05:13, *05:14, *05:15N?, *05:16?, *05:17N?, *05:20, *05:21?, *05:22?,
CAL Fluor Orange 560	**DQA1*02:01:01:01**		DQA1*02:01:01:02, *02:01:02, *02:02N-*02:10

Primermix II	Common*	*Well documented	*Rare
CAL Fluor Red 610	**DQB1*02:02:01:01**		DQB1*02:02:01:02-04, *02:02:02-12, *02:05? , *02:06, *02:10-*02:12, *02:13? , *02:15?-*02:25? , *02:26, *02:28-*02:47? , *02:49, *02:50, *02:51? , *02:52? , *02:54?-*02:46? , *02:58N? , *02:60? , *02:61? , *02:62, *02:64, *02:65, *02:66?-*02:71? , *02:73?-78? , *02:80, *02:84, *02:85?-*02:88? , *02:89:01/02, *02:90?-*02:94? , *02:95, *02:97, *02:100? , *02:101?, *02:103? , *02:104? , *02:110, *02:113, *02:116, *02:117, *02:120-*02:122, , *02:124, *02:126, *02:127, *02:129N? , *02:131, *02:137, *02:138, *02:139? , *02:140? , *02:141-*02:147, *02:150, *02:151? , *02:153, *02:156, *02:161, *02:162N, *02:163N? , *02:165, *02:167N
Quasar 670	**DQA1*05:01:01:01**, *05:03:01:01	DQA1*05:02	DQA1*05:01:01:02-04, *05:01:02/04/05/06, *0503:01:02, *05:03:02, *05:04, *05:07, *05:15N, *05:16, *05:18, *05:22
CAL Fluor Orange 560	**DQB1*03:02:01:01**	DQB1*03:02:02	DQB1*03:02:01:02-08, *03:02:04-06/08/09/11/12/15-31, *03:07, *0308, *0311, *03:18, *03:32, *03:45:01/02, , *03:62, *03:63, *03:64, *03:66N, *03:67, *03:68, *03:70, *03:81, *03:85, *03:106, *03:107, *03:125, *03:146, *03:287, *03:289, *03:295, *03:296, *03:298, *03:299, *03:308, *03:310N, *03:315, *03:320- *03:324, *03:333, *03:334N, *03:339N, *03:343, *03:344, *03:345, *03:348, *03:349, *03:362, *03:364, *03:367, *03:368, *03:369, *03:379, *03:383, *03:386, *03:388, *03:392, *03:403N, *03:409, *03:410, *03:412

IMGT Database 3.38.0

? no sequence information available for the primer binding site

* Common and well documented alleles from the CWD 2.0.0 catalogue (5)

BAG Diagnostics GmbH
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info@bag-diagnostics.com

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Fax: +49 (0) 6404 / 925-421
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7. WARNINGS AND PRECAUTIONS

The **FastQ CD kit** is designed for in vitro diagnostic use and should be used by properly trained, qualified staff only. All work should be performed using Good Laboratory Practices.

Biological material used for extraction of DNA, e.g. blood, 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.

Microbial contamination of the reagents while taking aliquots should be avoided. It is recommended to use sterile one way pipettes and tips. Reagents that look cloudy or show any signs of microbial contamination must not be used.

A Material Safety Data Sheet resp. a declaration on Material Safety Data Sheets (MSDS) is available to download at **www.bag-diagnostics.com**.

8. SPECIFIC PERFORMANCE CHARACTERISTICS

For the **FastQ CD** kit a performance evaluation study with a total of 90 pre-typed DNA samples were performed. The results from the study were compared to the results that were obtained with other CE certified typing reagents (amongst others serology, SSO, SSP) and/or sequencing. No discrepancies in the detection of the HLA DQ feature have been observed (100 % concordance).

Q Primermix I

DNA samples	Internal study total	Percentage concordance [%]
DQA1*02:01	26	100
DQA1*05:05	28	100
DQB1*02:01	14	100
DQA1*02:01, DQA1*05:05, DQB1*02:01 negativ	22	100
Total	**90**	**100**

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Fax: +49 (0) 6404 / 925-421
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Q Primermix II

DNA samples	Internal study total	Percentage concordance [%]
DQB1*03:02	17	100
DQA1*05:01	13	100
DQB1*03:02	20	100
DQB1*03:02, DQA1*05:01, DQB1*03:02 negativ	40	100
Total	**90**	**100**

9. LIMITATIONS OF THE METHOD

Because of the high susceptibility of the RT-PCR method for cross contaminations special care should be taken during DNA isolation. Validation tests in the course of the performance evaluation study of the **FastQ CD** kit have shown that a variation of the amount of DNA used for the amplification between 5 ng and 50 ng do not have a significant influence on the detection of the HLA-DQ alleles.

Extreme care should be taken to prevent contamination of the kit reagents and other laboratory materials and equipment with amplicons or DNA. Regular wipe tests (e.g. BAG Wipe Test, REF 7091) and negative controls with Aqua dest with each assay are strongly recommended.
In the negative control with Aqua dest. there must not be any fluorescent signal (Cq > N.A.). In the case of signal development in the negative control the PCR working place has to be decontaminated and the reagents have to be exchanged if necessary. All instruments (e.g. pipettes, realtime cyclers) must be calibrated according to the manufacturers instructions.

10. INTERNAL QUALITY CONTROL

Internal quality control of new lots of the **FastQ CD** kit can be performed using a combination of DNA specimens with known HLA type. An internal positive control for successful amplification is contained in the Q Primermixes I and II. Negative controls to detect possible contaminations are recommended. Use a PCR reaction without DNA (NTC) for this purpose.

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11. TROUBLESHOOTING

Symptom	Possible reason	Potential solution
Poor or no signal	Presence of an inhibitor in the PCR-reaction	Use fresh reagents
	Insufficient amount of DNA in the reaction	Repeat test with correct amount of DNA
	Wrong amplification parameters	Check PCR program
	Contaminated or degraded DNA	Check concentration and quality of the DNA Check DNA on a gel Repeat DNA isolation
	Degraded fluorescent probes or primers	Use fresh Q Primermix 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
	Incompatible or low quality RT-PCR plastics	Use compatible and high quality plastics
	Wrong signal calculation due to abnormal amplification signals during the initial cycles of the run	Application of corrective measures in the software (e.g. "apply fluorescence drift correction“ function from Bio-Rad or exclusion of the first five cycles from analysis)
	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 areas
Signal in the negative control	Contamination with DNA in the negative control	Repeat the test - decontaminate the workplace

12. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a trademark of Roche Molecular Systems Inc.

® Cal Fluor & Quasar Dyes are registered trademarks of LGC Biosearch Technologies

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13. EXPLANATION OF SYMBOLS USED ON THE LABELS

	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
CONT	Content, contains
GENOTYPING	Intended use: Typing of human genetic markers that are associated with diseases or pharmacogenetic reactions
IFU	Instruction for use
IVD	For in vitro diagnostic use
LOT	Batch code
PLEX MIX	Mastermix for RT-PCR
Q Primermix | CD I	Primermix number I for the detection of HLA-DQ-attributes with the FastQ CD kit
Q Primermix | CD II	Primermix number II for the detection of HLA-DQ-attributes with the FastQ CD kit
REF	Catalogue number

14. LITERATURE

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3. Husby et al., 2012. Journal of Pediatric Gastroenterology & Nutrition **54**:136-160
4. Beutler, E. et al., 1990. BioTechniques **9**:166
5. Mack et al., 2003, Tissue Antigens **81**:194-203

Instructions for use in other languages see
http://www.bag-diagnostics.com or contact us directly at info@bag-diagnostics.com
or phone +49 (0)6404-925-125

BAG Diagnostics GmbH
Amtsgerichtsstr. 1-5
35423 Lich/Germany
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