OLERUP SSP®
GENOMIC HLA TYPING

Technical Note for product:

Olerup SSP® DQA1*02,05; DQB1*02,0302 (101.903-24)

Background

Celiac disease (CD) is a chronic intestinal disorder resulting in villous atrophy and severe long-term complications. The disease occurs in genetically predisposed individuals in response to the ingestion of gluten and similar proteins in wheat, barley and rye and is characterized by the production of anti-tissue transglutaminase and anti-endomysial antibodies. It is usually assumed that CD affects approximately 1% of the general population in Europe and North America but a dedicated screening program identified an even higher frequency [1]. In contrast to other multifactorial disorders genetic testing in Celiac disease is of high value as the disease rarely develops in the absence of specific HLA alleles. The association can be explained by the fact that certain HLA-DQ heterodimers expressed on antigen-presenting cells specifically bind gluten-derived peptides and present them to T cells. The resulting T cell response then leads to the production of the disease specific antibodies and to the clinical symptoms.

It is well established that about 90-95% of patients in North America and Europe are positive for either the DQ2 or DQ8 serotype. In recent years it has been shown that the remaining CD patients mostly carry only one chain of the DQ2 heterodimer, and that CD occurs only exceptionally in the absence of any of these molecules [2, 3]. Given the strong association, HLA typing is recommended to be routinely used as a genetic test where the absence makes CD extremely unlikely [4].

While the HLA-DQ association with the risk of CD is widely accepted the practical usage and clinical implications of HLA typing results are still not clearly defined. Test concepts for CD associated alleles are therefore highly diverse and the interpretation of the results varies greatly. Often, only the classical allele combinations DQA1*05:01 DQB1*02:01 (cis), DQA1*05:05 02:01 DBQ1*03:01 02:02 (trans), and DQA1*03:01 DQB1*03:02 are regarded as "positive". Still, the prevalence of "atypical" combinations in the general population is high with either the α- or the β chain occurring on another than the usual haplotype or only one of the relevant chains being present. It has been shown that CD cannot be excluded in these individuals and that these cases account for about 6% of CD cases in Europe [3]. Furthermore, the implication of certain alleles in the development of the disease is not clear because they occur too rarely to be studied. E.g. DQB1*03:02 is usually associated with DQA1*03:01 due to the linkage disequilibrium. Since only about 5% of celiac patients carry DQB1*03:02 no information about the impact of other DQA1*03 walleles is available.

Any test for CD associated alleles should therefore provide highly detailed results allowing medical professionals full flexibility in interpretation. Olerup SSP AB has therefore designed the Olerup SSP® DQA1*02,05; DQB1*02,0302 test for CD associated alleles (product no. 101.903-24) with the aim to facilitate test interpretation while at the same time providing the highest level of specificity and information.

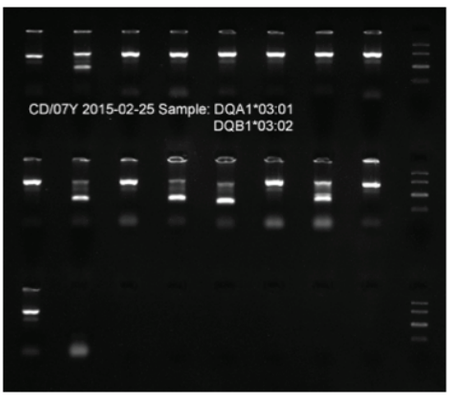
Alleles and Allele Combinations Discriminated for in the Olerup SSP® DQA1*02,05; DQB1*02,0302 Typing Kit (101.903-24)

DQA1 * 05:01 DQB1*02:01 (DQ2)
DQA1 * 05:05 DQB1*03:01 / DQA1*02:01 **DQB1*02:02** (DQ2)
DQA1 * 03 DQB1*03:02 (DQ8)
DQA1 * 05 DQB1*02 / DQA1*03 DQB1*03:02 (DQ2 + DQ8)
DQA1 * 05:01 X
DQA1 * 05:05 X
DQB1 * 02:01 X
DQB1 * 02:02 X
DQB1 * 03:02 X

α and β chains associated with the disease in bold
X = other allele currently not associated with the disease

The new test contains 17 mixes for the amplification and detection of all relevant DQA1 and DQB1 alleles plus a negative control well.

An improved combination of primers and mixes allows discrimination of DQ2 and DQ8 as well as the identification of the most commonly seen unusual allele combinations. Interpretation has been facilitated by a high overall resolution and a focus on common and well documented (CWD) alleles [5]. Furthermore the primer design has been optimized to result in larger products thus improving identification of positive wells. Example gel photos of kit performance are shown in image 1 and 2.



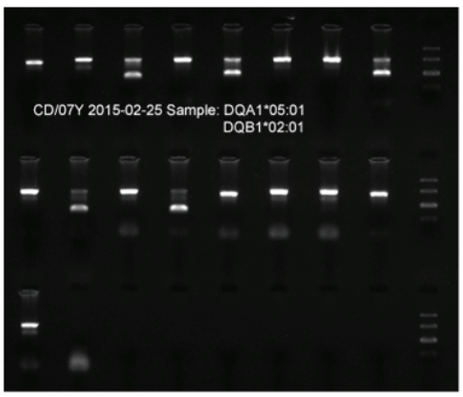


Image 1 and 2. Gel photos of two reference DNAs typed with the CD test; DQA1*03:01,DQB1*03:02 (DQ8) and DQA1*05:01,DQB1*02:01(DQ2) respectively.

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Result Interpretation and Data Handling

Interpretation of results can be easily achieved either by documentation provided with the kit or by using the supportive interpretational software Olerup SSP® version of Helmberg-SCORE™.
If using SCORE™: Under "Configuration > Typing Options" choose "Split into common alleles and rare combinations". As all CD associated alleles are common alleles, they will be listed separately if present in the sample.
Please note that alleles associated with CD are positively detected by the test or excluded if not present. The test will not perform a full typing and therefore can list additional alleles that might or might not be present. Negative results are therefore characterized by the complete absence of any of the CD associated alleles. A positive test result will list the DQA1 and DQB1 alleles concordant with the SSP pattern observed.

Expected results

Table 1 describes expected reactivity patterns for the (groups of) alleles that the kit is able to detect and separate.

Table 1: Expected results for targeted DQA1 and DQB1 alleles.

DQA1 alleles	DQB1 alleles	Positive DQA1 wells	Positive DQB1 wells
*05:01	*02:01 (DQ2)	3, 5	8, 10, 12
(*02:01) *05:05*	*02:02 (DQ2) (*03:01)	1 3, 5, 6	8, 9, 12 10, 15, 17
*03	*03:02 (DQ8)	2	10, 12, 13, 15
*05:01		3, 5	
*05:05		3, 5, 6	
*02:01		1	
*03		2	
	*02:01		8, 10, 12
	*02:02		8, 9, 12
	*03:01		10, 15, 17
	*03:02		10, 12, 13, 15

Further Reading

[1] Catassi C, Kryszak D, Louis-Jacques O, et al.
Detection of Celiac disease in primary care: A multicenter case-finding study in
North America. Am J Gastroenterol 2007;102:1454–60.

[2] Megiorni F, Mora B, Bonamico M, Barbato M, Nenna R, Maiella G, Lulli P, Mazzilli MC. HLA-DQ and risk gradient for celiac disease. Human Immunology 2009; 70: 55-59

[3] Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: Results from the European Genetic Cluster on Coeliac Diesease. Hum Immunol 2003;64:469–77.

[4] European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of
Coeliac Disease; Husby et al; JPGN _ Volume 54, 2012

[5] S. J. Mack1, P. Cano2, J. A. Hollenbach1 et al.
Common and well-documented HLA alleles: 2012 update to the CWD catalogue. Tissue Antigens, 2013, 81, 194–203

[6] Margaritte-Jeannin P, Babron MC, Bourgey M, et al.
HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease. Tissue Antigens. 2004 Jun;63(6):562-7.

[7] Megiorni F, and Pizzuti A. HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. Journal of Biomedical Science 2012, 19:88

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Frequently Asked Questions

Can the kit discriminate DQ2 and DQ8?

Yes. The kit can discriminate DQ2, DQ8 and DQ2 + DQ8 allele combinations.

Can the kit detect DQA1*03:01 in DQ8?

The kit can detect DQA1*03. Because of the linkage disequilibrium DQB1*03:02 (DQ8) is usually always found in combination with DQA1*03:01 in CD patients. As DQ8 is only present in a small percentage of cases other DQA1*03 alleles occur so rarely that no data is available with regards to their impact on the disease. We have therefore decided not to resolve DQA1*03 any further.

Why does my result show alleles that are not described in CD?

Since the kit is not designed to perform a complete typing of the DQA1 and DQB1 loci some alleles cannot be excluded from the result. These alleles depend on the genotype of the patient and have no meaning in the interpretation of the test.

What is a positive result?

The test will give rise to distinctive patterns with all alleles presently associated with CD. The clinical impact of the different alleles and combinations is still a matter of debate though and can also depend on the population studied [6]. Interpretation especially of unusual allele combinations should therefore be done by medical professionals. Further information on the risk for CD of different genotypes can be found in [7].

What is the accuracy of the test?

The test has been designed based on common and well documented alleles (CWD) [5]. As other alleles occur with a frequency of less than 1:1000 false positive results can be expected to be extremely rare. The accuracy of negative results (all relevant CD associated alleles are excluded by the test) is 100%.

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