

HLA-DQB1 Hybridization Assay

DELFLIA[®]

Diabetes Reagents

This assay is intended for scientific research use only. Not for use in diagnostic procedures.

This assay should only be used by investigators certified by Wallac Oy.

These instructions for use apply to the following reagents:

	<u>Product number</u>	<u>Description</u>	<u>DQB1 alleles recognized by the probe</u>
<u>Basic set</u>	AD0127 AD0128 AD0129 AD0130 AD0131 AD0132	Eu-DQB1*0602, 0603 probe Sm-DQB1*0603, 0604 probe Tb-DQB1 Control probe Eu- DQB1*0302 probe Sm-DQB1*0301 probe Tb-DQB1*02 probe	*0602-3, 0607-8, 0610-11, 0613-15, 06042 *0603-4, 0607-8, 0614, 0617 all alleles *0302, 0304-5, 0307-8 *0301, 0304, 0309 *0201-3
<u>Extension set</u>	AD0150 AD0151 AD0152 AD0153 AD0154 AD0155	Eu-DQB1*0601 probe Sm-DQB1*04, 05 probe Tb-DQB1*0301, 0303 probe Eu-DQB1*04 probe Sm-DQB1*05011 probe Tb-DQB1*0502, 0504 probe	*0601 *0305, 04, 05 *0301, 0303, 0306, 0309-10 *03051, 04 *05011 *0502, 0504, 0610
<u>Controls</u>	AD0137 AD0156	Bio-DQB1 Hybridization control Bio-DQB1 extension Hybridization control	<u>Complementarity to DQB1-probes</u> Complementary to AD0127-AD0132 probes Complementary to AD0150-AD0155 probes

INTRODUCTION

The basic set of five sequence specific oligonucleotide probes contains reagents to define the presence of HLA-DQB1*02, 0302, 0301, 0602 and 0603 alleles. The extension set contains six sequence specific oligonucleotide probes defining the presence of HLA-DQB1*0303, 04, 05 and 0601 alleles. The additional alleles recognized by these probes are presented above.

A consensus sequence specific probe (Tb-DQB1 Control) is included in the basic set to control the success of the PCR¹ (polymerase chain reaction) amplification. The biotinylated hybridization controls containing the complementary sequences of the probes can be used as artificial positive samples to control the success of the hybridization reaction.

The assay is based on DNA amplification of HLA-DQB1 alleles and lanthanide(III) chelate labeled DNA probes specific for the alleles described above. Analysis of the amplified DNA is performed by collecting the biotin labeled DNA fragments onto streptavidin-coated microtitration wells, denaturing the collected fragments, and hybridizing them with lanthanide(III) chelate labeled probes (1).

Due to their unique properties, lanthanide(III) chelates provide a sensitive label technology. The DELFIA[®] technology utilizes efficient labeling of biomolecules with lanthanide(III) chelates and highly sensitive time-resolved fluorometric detection after a dissociative fluorescence enhancement (2,3). The oligonucleotide probes are labeled with Eu(III) / Sm(III) / Tb(III) chelate of 2,2',2'',2'''-{{4-[2-(4-isothiocyanatophenyl)ethyl]pyridine-2,6-diyl}bis(methylenenitrilo)}tetrakis(acetate). The labeled oligonucleotides as such are non-fluorescent, but after hybridization, the lanthanides are dissociated to form highly fluorescent Eu(III), Sm(III) and Tb(III) chelates in the DELFIA Enhancement Solution and the DELFIA Enhancer. Since the technology makes it possible to hybridize three DNA probes in one reaction, the whole set can be hybridized in two reactions.

The analysis with the probes requires a DNA amplification step and the amplification product should be biotin labeled in order to collect the product onto streptavidin-coated wells. The amplification of the DQB1 alleles can be carried out by PCR using the following primers, 5' primer: GCA TGT GCT ACT TCA CCA ACG, 3' primer: Bio-CCT TCT GGC TGT TCC AGT ACT. The reactions can be performed on PCR plates in a reaction volume of 50 µL (100 µL for both sets) using a blood spot or isolated genomic DNA as template. The optimal performance of PCR is reached with heat-treated blood spots. The PCR parameters should be established in each individual laboratory.

VIAL CONTENT

Labeled reagents are supplied as ready-for-use solutions in 20 mmol/L Tris-HCl buffered 50 mmol/L sodium chloride (NaCl). One probe vial contains 10 µg of labeled

¹ Since the PCR process is covered by patents owned by Hoffman-LaRoche, Inc., the process shall not be used without a license. The Buyer shall sign an undertaking stating that they have the license and agree to indemnify and hold the Seller harmless in case of any possible actions, suits or proceedings based on a claim of unauthorized use of the process.

oligonucleotide probe in a concentration of 10 µg/mL, and a control vial 100 µL of biotinylated control.

STORAGE

Store labeled oligonucleotide as such at -20°C. We recommend aliquoting the oligonucleotides for storage. Do not store labeled oligonucleotides in DELFIA Hybridization Buffer, phosphate buffer or any other buffer that contains chelating agents.

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE PRODUCT

The DELFIA system requires the following items, which are available from PerkinElmer Life Sciences or its distributors.

1. Time-resolved fluorometer -1420 VICTOR™ Multilabel Counter
2. Automatic shaker - DELFIA Plateshake (prod. no. 1296-003/004)
3. Automatic washer - DELFIA Platewash (prod. no. 1296-026)
4. DELFIA system reagents:
 - DELFIA Streptavidin Microtitration Strips (prod. no. 4009-0010, 10 plates/package)
 - DELFIA Hybridization Buffer (prod. no. 4006-0010)
 - DELFIA Wash Concentrate (prod. no. 1244-114)
 - DELFIA Enhancement Solution (prod. no. 1244-105)
 - DELFIA Enhancer (prod. no. C500-100)
5. Pipette for dispensing the DELFIA Enhancement Solution and the DELFIA Enhancer - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) and 2.5 mL Combitips (prod. no. 1296-017). The DELFIA Plate Dispense (prod. no. 1296-041) can be used for dispensing the DELFIA Enhancement Solution).

In addition to the DELFIA system the following are required:

- reagents and equipment to perform DQB1-gene amplification
- +37°C incubator for incubating plates
- +45°C incubator for warming up the wash solution
- precision pipettes
- denaturation solution: 20 mmol/L NaOH
- distilled water
- adhesive tape or other cover for plates to avoid contamination by PCR products during collection and evaporation of hybridization solutions during incubation at +37°C

WARNINGS AND PRECAUTIONS

This assay is intended for scientific research use only. Not for use in diagnostic procedures.

Disposal of all waste should be in accordance with local regulations.

DQB1-TRF-HYBRIDIZATION PROTOCOL

1. Collection of the DNA amplification product onto streptavidin coated wells:

1.1. Basic set (AD0127-AD0132)

Pipette 10 μ L of distilled water into four streptavidin-coated microtitration wells for following the hybridization background (e.g. to the first four wells of the plate, since hybridization is done in duplicates with two hybridization solutions).

Extension set (AD0150-AD0155)

Pipette 10 μ L of distilled water into six streptavidin-coated microtitration wells for following the hybridization background (e.g. to the first six wells of the plate, since hybridization is done in duplicates with three hybridization solutions).

Both sets

Pipette 10 μ L of distilled water into four microtitration wells on two streptavidin-coated plates for following the hybridization background (e.g. to the first four wells of the plates, since hybridization is done in duplicates with two hybridization solutions on each plate).

1.2. Basic set

Pipette 10 μ L of diluted (1:100 in hybridization buffer) hybridization control AD0137 into four wells (hybridized with two hybridization solutions in duplicate) for following the success of the hybridization reaction.

Extension set

Pipette 10 μ L of diluted (1:100 in hybridization buffer) hybridization control AD0156 into four wells (hybridized with two hybridization solutions in duplicate) and 10 μ L of diluted hybridization control AD0137 into the next two wells (hybridized with control probe solution in duplicate) for following the success of the hybridization reaction.

Both sets

Pipette 10 μ L of diluted (1:100 in hybridization buffer) hybridization control AD0137 into four wells on the first plate and 10 μ L of diluted hybridization control AD0156 into four wells on the second plate (hybridized with two hybridization solutions on each plate in duplicate) for following the success of the hybridization reaction.

1.3. Basic set

Pipette 10 μ L of each amplification reaction product into four wells (each sample is hybridized with two hybridization solutions in duplicates). We recommend the use of a PCR background sample (no template in PCR) in every PCR run to check for possible contamination. This contamination control should be run in the same way as the samples in the hybridization assay.

Extension set

Pipette 10 μ L of each amplification reaction product into six wells (each sample is hybridized with three hybridization solutions in duplicates). We recommend the use of a PCR background sample (no template in PCR) in every PCR run to check for possible contamination. This contamination control should be run in the same way as the samples in the hybridization assay.

Both sets

Pipette 10 µL of each amplification reaction product into four wells on two plates (each sample is hybridized with two hybridization solutions on each plate in duplicates). We recommend the use of a PCR background sample (no template in PCR) in every PCR run to check for possible contamination. This contamination control should be run in the same way as the samples in the hybridization assay.

Pipetting chart for basic DQB1 set. If both probe sets are hybridized simultaneously the pipetting is done similarly on two plates.

1	2	3	4	5	6	7	8	9	10	11	12	Strip
Bg A	Bg A	Bg B	Bg B	Ctrl A	Ctrl A	Ctrl B	Ctrl B	PCR A	PCR A	PCR B	PCR B	A
S1 A	S1 A	S1 B	S1 B	S2 A	S2 A	S2 B	S2 B	S3 A	etc.			B
Bg for hybridization background, Ctrl for hybridization control, PCR for PCR background sample, S1, S2, etc. for unknown PCR samples												C etc.

Pipetting chart for extension set which requires an additional hybridization solution containing the Tb-DQB1 Control probe.

1	2	3	4	5	6	7	8	9	10	11	12	Strip
Bg A	Bg A	Bg B	Bg B	Bg C	Bg C	Ctrl A	Ctrl A	Ctrl B	Ctrl B	Ctrl C	Ctrl C	A
PCR A	PCR A	PCR B	PCR B	PCR C	PCR C	S1 A	S1 A	S1 B	S1 B	S1 C	etc.	B
Bg for hybridization background, Ctrl for hybridization control, PCR for PCR background sample, S1, S2, etc. for unknown PCR samples												C etc.

1.4. Add 50 µL of DELFIA Hybridization Buffer into each well.

1.5. Incubate the plate for 30 minutes at room temperature (+20 - +25°C) using the DELFIA Plateshake (slow shaking).

2. Preparation of hybridization solutions

Each sample should be hybridized with two hybridization solutions (A and B) in duplicate. Prepare the hybridization solutions A and B as stated below. Please note that the hybridization solutions should be used as soon as possible (within 30 minutes even when stored on ice).

Add the correct volume of each probe to the hybridization buffer to prepare the respective hybridization solutions (A and B).

DQB1 basic set: Hybridization solutions for one plate (96 wells):

Product no.	Description	A	B
	Hybridization buffer	6 mL	6 mL
AD0127	Eu- DQB1*0602, 0603 probe	120 ng	-
AD0128	Sm- DQB1*0603, 0604 probe	120 ng	-
AD0129	Tb- DQB1 Control probe	120 ng	-
AD0130	Eu-DQB1*0302 probe	-	90 ng
AD0131	Sm- DQB1*0301 probe	-	90 ng
AD0132	Tb- DQB1*02 probe	-	60 ng

DQB1 extension set: Hybridization solutions for one plate (96 wells)

Product no.	Description	A	B	C
	Hybridization buffer	4 mL	4 mL	4 mL
AD0150	Eu- DQB1*0601 probe	80 ng	-	-
AD0151	Sm- DQB1*04, 05 probe	80 ng	-	-
AD0152	Tb- DQB1*0301, 0303 probe	80 ng	-	-
AD0153	Eu-DQB1*04 probe	-	80 ng	-
AD0154	Sm- DQB1*05011 probe	-	80 ng	-
AD0155	Tb- DQB1*0502, 0504 probe	-	80 ng	-
AD0129	Tb- DQB1 Control probe	-	-	80 ng

Both sets: Hybridization solutions for two plates (2 x 96 wells).

Product no.	Description	A1	B1	A2	B2
	Hybridization buffer	6 mL	6 mL	6 mL	6 mL
AD0127	Eu- DQB1*0602, 0603 probe	120 ng	-	-	-
AD0128	Sm- DQB1*0603, 0604 probe	120 ng	-	-	-
AD0129	Tb- DQB1 Control probe	120 ng	-	-	-
AD0130	Eu-DQB1*0302 probe	-	90 ng	-	-
AD0131	Sm- DQB1*0301 probe	-	90 ng	-	-
AD0132	Tb- DQB1*02 probe	-	60 ng	-	-
AD0150	Eu- DQB1*0601 probe	-	-	120 ng	-
AD0151	Sm- DQB1*04, 05 probe	-	-	120 ng	-
AD0152	Tb- DQB1*0301, 0303 probe	-	-	120 ng	-
AD0153	Eu-DQB1*04 probe	-	-	-	120 ng
AD0154	Sm- DQB1*05011 probe	-	-	-	120 ng
AD0155	Tb- DQB1*0502, 0504 probe	-	-	-	120 ng

(The volumes can be adjusted if the number of plates / strips is altered.)

3. Denaturation of double stranded DNA

- 3.1. Prepare wash solution by diluting the DELFIA Wash Concentrate 25-fold with distilled water. Wash solution remains stable for 2 weeks at +2 - +25°C in a sealed container.
- 3.2. Wash the plate 3 times with wash solution
- 3.3. Add 150 µL of 20 mmol/L NaOH into each well.
- 3.4. Shake the plate slowly on the DELFIA Plateshake for 5 minutes at room temperature.
- 3.5. Wash the plate 3 times with wash solution.

4. Hybridization

4.1. Basic set

Add 100 µL of the respective hybridization solutions (A or B; prepared as stated in section 2 above) into two wells of each sample on the plate (A into wells 1 and 2, B into wells 3 and 4, etc.; see pipetting chart in section 1).

Extension set

Add 100 µL of the respective hybridization solutions (A, B or C; prepared as stated in section 2 above) into two wells of each sample on the plate (A into wells 1 and 2, B into wells 3 and 4, C into wells 5 and 6 etc.; see pipetting chart in section 1).

Both sets

Add 100 µL of the respective hybridization solutions (A or B; prepared as stated in section 2 above) into two wells of each sample on the plate (A1 into wells 1 and 2 on plate with basic set, B1 into wells 3 and 4 on plate with basic set, A2 into wells 1 and 2 on plate with extension set, B2 into wells 3 and 4 on plate with extension set, etc.; see pipetting chart in section 1).

- 4.2. Seal the plate with an adhesive tape.
- 4.3. Incubate for 2 hours (\pm 10 minutes) at +37°C (\pm 1°C).

5. Stringent washes

- 5.1. Wash the plate 6 times with wash solution preheated to +45°C (\pm 1°C).

6. Enhancement

- 6.1. Add 200 µL of the DELFIA Enhancement Solution into each well.
- 6.2. Incubate the plate for 30 minutes at room temperature using the DELFIA Plateshake (slow shaking).

6.3. Basic set

- Measure Eu / Sm fluorescence with a time-resolved fluorometer. Select the Diabetes DQB1EuSm MultiCalc[®] program for automatic measurement and result calculation or calculate the results manually.
- Add 50 µL of the DELFIA Enhancer into each well.
- Shake the plate slowly on the DELFIA Plateshake for 5 minutes.
- Measure Tb fluorescence with a time-resolved fluorometer. Select the Diabetes DQB1Tb MultiCalc program for automatic measurement and result calculation or calculate the results manually.

Extension set

- Measure Eu / Sm fluorescence with a time-resolved fluorometer. Select the Diabetes DQB1extEuSm MultiCalc program for automatic measurement and result calculation or calculate the results manually.
- Add 50 µL of the DELFIA Enhancer into each well.
- Shake the plate slowly on the DELFIA Plateshake for 5 minutes.
- Measure Tb fluorescence with a time-resolved fluorometer. Select the Diabetes DQB1extTb MultiCalc program for automatic measurement and result calculation or calculate the results manually.

Both sets

Measure fluorescence with a time-resolved fluorometer. For automatic measurement and result calculation select appropriate Diabetes MultiCalc program from the list below. After each Eu/Sm measurement add 50 µL of the DELFIA Enhancer into each well and shake the plate slowly on the DELFIA Plateshake for 5 minutes. Measure the plates in the following order:

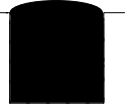
	<u>Plate</u>	<u>Program</u>
1.	plate(s) with basic set probes	DQB1EuSm
2.	plate(s) with basic set probes	DQB1Tb
3.	plate(s) with extension set probes	2xDQB1EuSm
4.	plate(s) with extension set probes	2xDQB1Tb

or calculate the results manually.

NOTE: The 2xDQB1 programs pick the result for the control probe from the previous measurement. No other DQB1 MultiCalc measurement should thus be allowed between the measurements described.

PROCEDURAL NOTES

1. The labeled probes are stable under normal hybridization conditions. For prolonged storage at -20°C we recommend aliquoting the probes and control. Avoid unnecessary storage at room temperature.
2. Avoid exposure of lanthanide labeled probes to pH lower than 7 and EDTA concentration higher than 200 µmol/L.
3. When washing the wells, ensure that each well is filled up completely to the top edge as shown in the figure. After washing the strips, check that the wells are dry.



Pay special attention to rinsing the washing device thoroughly with distilled water after the hybridization washes have been completed. For detailed information on the cleaning and maintenance of the washing device, please refer to the DELFIA Platewash manual.

4. The avoidance of lanthanide contamination and resulting high fluorescent background demands high standard pipetting and washing techniques. Avoid contaminating pipettes with Eu / Sm / Tb-labeled reagents.
5. The DELFIA Enhancement Solution should be dispensed using only the recommended Eppendorf Multipipette after the Combitip has first been flushed with Enhancement Solution according to the instructions for use. The same Combitip must not be used for pipetting any other reagent.

When using the DELFIA Plate Dispense, please refer to the manual.

EXPECTED VALUES AND INTERPRETATION OF RESULTS

Interpretation of the results follows the hybridization pattern of DQB1 probes presented in table 1.

The results illustrated in the tables below were obtained, when samples with known HLA-DQB1 genotypes were assayed according to the protocol described. The control probe cps (counts per second) and S/N (signal-to-noise; specific signal divided by the hybridization background signal) ratio reflect the amplification level (Tables 2a and 2b). A S/N ratio >100 obtained with the control probe can generally be regarded as an adequate amplification level. Occasionally, lower S/N ratios can be accepted when combined with clearly negative or positive results with all the other probes without the need of repeating PCR and hybridization assay (i.e. sample no 17).

In addition to S/N ratio calculations, we suggest to equalize the different amplification levels by comparing the S/N ratio of each individual allele-specific probe in a particular sample to the S/N ratio of the corresponding control probe (reference value = 100%) and express the results in percentages (Tables 3a and 3b). Thus a probe-specific variation typical for positive samples can be obtained to facilitate the discrimination into the two categories (positive/negative). Pay attention to the interpretation of DQB1*0602, 0603 probe results, since there is a tendency of this probe to cross-react with the DQB1*02, 02 genotype, also probes DQB1*0301, 0303 and DQB1*04 tend to cross-react.

The target values of intra-assay (within run) variation for a positive sample are given below and grouped by the S/N ratio. When the signals of duplicate samples (positive sample) differ from each other more than 25%, we recommend repeating the hybridization assay.

S/N	CV %
< 50	< 20
> 50	< 15

Sample no	Genotype	Eu-0602, 0603		Sm-0603, 0604		Eu-0302		Sm-0301		Tb-02		Tb Control	
		cps	S/N	cps	S/N	cps	S/N	cps	S/N	cps	S/N	cps	S/N
1	602, 602	829228	287.0	203	1.0	5588	4.6	415	2.2	3999	2.5	1043376	752.3
2	603, 603	913504	316.2	17425	86.7	2127	1.7	290	1.6	4029	2.5	724701	522.5
3	604, 604	19404	6.7	24626	122.5	1838	1.5	534	2.9	5597	3.5	922469	665.1
4	02, 02	59622	20.6	565	2.8	5070	4.2	705	3.8	837627	523.2	185772	133.9
5	301, 301	6620	2.4	246	1.2	2422	2.0	30837	165.8	2713	1.7	662908	477.9
6	302, 302	6999	2.4	205	1.0	41762	34.3	330	1.8	3700	2.3	342609	247.0
7	302, 604	19125	6.6	15012	74.7	118099	97.1	621	3.3	11904	7.4	894697	645.1
8	301, 302	33966	11.8	398	2.0	138484	113.9	44968	241.8	26626	16.6	952350	686.6
9	02, 02	51521	17.8	820	4.1	4781	3.9	977	5.3	811965	507.2	172713	124.5
10	02, 02	14694	5.1	272	1.4	2834	2.3	334	1.8	893586	558.1	153490	110.7
11	02, 302	23412	8.1	242	1.2	200018	164.5	405	2.2	973051	607.8	813739	586.7
12	02, 02	16952	5.9	555	2.8	6875	5.7	658	3.5	917743	573.2	614867	443.3
13	02, 604	12030	4.2	22246	110.7	14150	11.6	727	3.9	1081085	675.3	904995	652.5
14	02, 602	584074	202.2	228	1.1	3060	2.5	268	1.4	824872	515.2	533274	384.5
15	02, 301	62629	21.7	491	2.4	12640	10.4	40515	217.8	888954	555.2	580404	418.5
16	02, 603	716938	248.2	26499	131.8	7892	6.5	789	4.2	1086508	678.6	992393	715.5
17	603	159500	55.2	1323	6.6	2826	2.3	221	1.2	1631	1.0	90371	65.2
Bg (hybridization)		2889	1.0	201	1.0	1216	1.0	186	1.0	1601	1.0	1387	1.0
Bg (PCR)		2241	0.8	309	1.5	1914	1.6	297	1.6	2128	1.3	3574	2.6
Hybridization control		806765	279.9	35791	178.1	976157	802.8	23696	127.4	875282	546.7	649679	468.6

Table 2a.

Basic set of probes. The time-resolved fluorescence signals (cps) and corresponding signal-to-noise ratios (S/N) obtained in a study performed at Wallac Oy.

Sample no	Genotype	Eu-0601		Sm-04, 05		Tb-0301, 0303		Eu-04		Sm-05011		Tb-0502, 0504		Tb Control	
		cps	S/N	cps	S/N	cps	S/N	cps	S/N	cps	S/N	cps	S/N	cps	S/N
18	04, 501	4526	5.6	43519	195	25364	22.0	814466	1451	66131	272	1040	1.1	297919	390
19	02, 501	2385	2.9	24252	108	1406	1.2	2458	4.4	45341	186	1694	1.8	357907	468
20	302, 303	525	0.6	224	1.0	409059	354	1594	2.8	245	1.0	897	0.9	1063935	1392
21	303, 604	694	0.9	297	1.3	316012	274	1967	3.5	999	4.1	968	1.0	851544	11114
22	301, 04	469	1.0	5499	22	349842	274	441530	991	731	2.9	900	1.0	452283	503
23	02, 303	635	1.3	237	1.0	370522	290	1965	4.4	289	1.2	868	1.0	817194	909
24	302, 502	1493	3.9	26545	112	24923	30.3	1439	3.6	293	1.3	19139	23.8	945384	572
25	301, 501	2565	6.7	30781	129	661500	805	6117	15.1	46982	202	2893	3.6	729596	442
26	304, 04	658	1.7	8923	37.6	408223	496	759819	1878	347	1.5	976	1.2	1118852	677
27	02, 04	588	1.0	12174	53.0	4352	3.9	866200	1536	1043	4.5	992	1.1	526136	444
28	04, 503	816	1.4	50405	213	12053	12.2	3445986	6311	468	2.0	980	1.1	618159	624
29	303, 501	2176	3.8	30300	128	490854	496	1863	3.4	38734	165	959	1.1	870151	878
30	04, 502	3966	6.9	52510	222	4056	4.1	1561342	2860	340	1.4	37575	42.4	378614	382
31	02, 305	4187	6.5	51457	249	23878	18.3	3737714	4341	775	3.7	1244	1.1	839570	521
32	04, 04	867	1.5	56284	245	3431	3.1	3940495	6987	595	2.5	1193	1.3	788887	666
Bg (hybridization)		577	1.0	230	1.0	1109	1.0	564	1.0	234	1.0	936	1.0	1184	1.0
Bg (PCR)		1264	2.2	6435	28.0	69826	63.0	38748	68.7	8253	35.3	1159	1.2	176075	149
Hybridization control		1542533	2673	23842	104	726010	655	1395388	2474	15673	67.0	197898	211	576059	486

Table 2b.

Extension set of probes.
The time-resolved fluorescence signals (cps) and corresponding signal-to-noise ratios (S/N) obtained in a study performed at Wallac Oy.

Table 3a. The results of Table 2a expressed as percentages from reference (Control probe). The positive results are bolded.

Sample no.	Genotype	% from reference					
		0602, 0603	0603, 0604	0302	0301	02	Control
1	602, 602	38	0	1	0	0	100
2	603, 603	61	17	0	0	0	100
3	604, 604	1	18	0	0	1	100
4	02, 02	15	2	3	3	391	100
5	301, 301	0	0	0	35	0	100
6	302, 302	1	0	14	1	1	100
7	302, 604	1	12	15	1	1	100
8	301, 302	2	0	17	35	2	100
9	02, 02	14	3	3	4	407	100
10	02, 02	5	1	2	2	504	100
11	02, 302	1	0	28	0	104	100
12	02, 02	1	1	1	1	129	100
13	02, 604	1	17	2	1	103	100
14	02, 602	53	0	1	0	134	100
15	02, 301	5	1	2	52	133	100
16	02, 603	35	18	1	1	95	100
17	603	85	10	4	2	2	100
Bg (PCR)		31	58	62	62	50	100
Hybridization control		60	38	171	27	117	100

Table 3b. The results of Table 2b expressed as percentages from reference (Control probe). The positive results are bolded.

Sample no.	Genotype	% from reference						
		0601	04, 05	0301, 0303	04	05011	0502, 0504	Control
18	04, 501	1	50	6	372	70	0	100
19	02, 501	1	23	0	1	40	0	100
20	302, 303	0	0	25	0	0	0	100
21	303, 604	0	0	25	0	0	0	100
22	301, 04	0	4	55	197	1	0	100
23	02, 303	0	0	32	0	0	0	100
24	302, 502	1	20	5	1	0	4	100
25	301, 501	2	29	182	3	46	1	100
26	304, 04	0	6	73	277	0	0	100
27	02, 04	0	10	1	299	1	0	100
28	04, 503	0	34	2	1012	0	0	100
29	303, 501	0	15	57	0	19	0	100
30	04, 502	2	58	1	748	0	11	100
31	02, 305	1	48	4	833	1	0	100
32	04, 04	0	26	0	1008	0	0	100
Bg (PCR)		2	19	42	46	24	1	100
Hybridization control		549	21	135	509	14	43	100

WARRANTY

Purchase of the product gives the purchaser the right to use this material in his own research, development, and investigational work. The product is not to be injected into humans or used for diagnostic procedures. Wallac Oy reserves the right to discontinue or refuse orders to any customer who plans to use these products for any other purposes.

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All information supplied with the product and technical assistance given is believed to be accurate, but it remains the responsibility of the investigator to confirm all technical aspects of the application. We appreciate receiving any additions, corrections, or updates to information supplied to the customer.

REFERENCES

1. Sjöroos, M., Iitiä, A., Ilonen, J., Reijonen, H. and Lövgren, T. (1995): Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *BioTechniques* **18**, 870-877.
2. Soini, E. and Kojola, H. (1983): Time-resolved fluorometer for lanthanide chelates. A new generation of non-isotopic immunoassays. *Clin. Chem.* **29**, 65-68.
3. Hemmilä, I., Dakubu, S., Mukkala, V.-M., Siitari, H. and Lövgren, T. (1984): Europium as a label in time-resolved immunofluorometric assays. *Anal. Biochem.* **137**, 335-343.

Additional literature:

Ilonen, J., Reijonen, H., Herva, E., Sjöroos, M., Iitiä, A., Lövgren, T., Veijola, R., Knip, M., Åkerblom, H-K. and the childhood diabetes in Finland (DiMe) study group (1996): Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. *Diabetes Care* **19**, 795-800.

Nejentsev, S., Koskinen, S., Sjöroos, M., Reijonen, H., Schwartz, E.I., Kovalchuk, L., Sochnev, A., Adojaan, B., Podar, T., Knip, M., Simell, O., Koskenvuo, M., Åkerblom, H.K. and Ilonen, J. (1998): Distribution of insulin-dependent diabetes mellitus (IDDM) -related HLA-alleles correlates with the difference in IDDM incidence in four populations of the Eastern Baltic region. *Tissue Antigens* 52473-477.

Nejentsev, S., Sjöroos, M., Soukka, T., Knip, M., Simell, O., Lövgren, T. and Ilonen, J. (1999): Population-based genetic screening for the estimation of type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. *Diabetic Medicine* **16**, 985-992.

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