

Comparison of DNA purification on the EZ1[®] Advanced XL and the EZ2[®] Connect MDx

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Introduction

In this application note, we compare performance of the EZ1[®] DSP DNA Blood Kit in combination with the EZ1 Advanced XL or the EZ2[®] Connect MDx to determine whether similar yields and purities are obtained for the purified genomic DNA purified from human whole blood samples.

The EZ2 Connect MDx is the recent successor of the successful EZ1 instrument and provides additional and improved features. The new instrument automates all the kits that are available for the EZ1 instruments and provides an additional load check procedure to ensure that all necessary reagents and consumables have been loaded and are not expired before the run is performed. Ready-to-use application protocols are available to automate the purification of EZ1 DSP kits as well as EZ1, EZ1&2[™] and EZ2 Kits.

Human leukocyte antigen (HLA) loci were analyzed in this comparison, as typing of this large gene complex is routinely used in laboratories worldwide. Two established techniques were employed for the typing, using either sequence-specific oligonucleotide (SSO) probes or an multiplex real-time PCR approach.

Our results indicate that the EZ2 Connect MDx performs as well as the EZ1 Advanced XL for genomic DNA purification.

Materials and methods

Genomic DNA was extracted from 102 fresh human whole blood samples (stored at room temperature) using the EZ1 DSP DNA Blood Kit (48) (cat. no. 62124) according to the kit instructions with 350 µL sample volume and 100 µL elution volume. Two aliquots of each sample were taken to enable purifications on EZ1 Advanced XL and the EZ2 Connect MDx.

Purified DNA (100 samples on each instrument) was subjected to analysis of human leukocyte antigen (HLA) typing tests using two different technical approaches. Reverse SSO DNA-typing assay technology for one class I locus was performed using the LABType[®] XR Class I A Locus Typing Test (cat. no. RSOX1AT, One Lambda, Inc.) and for one class II locus using the LABType XR Class II DRB1 Locus Typing Test (cat. no. RSOX2B1T, One Lambda, Inc.) with a total of 20 samples per locus.

Multiplex real-time PCR-based HLA typing was performed for 11 loci using hydrolysis probes in plate format using

the Multiplex QTYPE11® plate (cat. no. 201.701-10-RUO, CareDx Lab Solutions) with 10 samples (1 sample per plate).

All samples were assessed for DNA yield and purity using analytical methods: in particular, the UV spectrum, including the ratio of absorbance at 260 nm and 280 nm, was determined using the NanoDrop® 2000 instrument (Thermo Scientific).

Results

Results of the functional analysis on SSO DNA typing for HLA-DRB1 are summarized in Table 1.

As Table 1 shows, we observed 100% concordant results between the EZ2 Connect MDx and EZ1 Advanced XL instruments for each sample.

Table 1. Functional results for reverse sequence-specific oligonucleotide DNA-typing assay for one class II locus (HLA-DRB1)

Sample ID	EZ1 Advanced XL		EZ2 Connect MDx	
1	03:01	04:01	03:01	04:01
2	03:01	11:01	03:01	11:01
3	03:01	15:01	03:01	15:01
4	07:01	11:04	07:01	11:04
5	08:04	12:01	08:04	12:01
6	15:01	15:02	15:01	15:02
7	15:01	15:01	15:01	15:01
8	01:01	03:01	01:01	03:01
9	04:05	11:01	04:05	11:01
10	04:01	15:03	04:01	15:03
11	11:01	15:03	11:01	15:03
12	01:02	07:01	01:02	07:01
13	03:02	11:01	03:02	11:01
14	13:02	14:01/ 14:54	13:02	14:01/ 14:54
15	04:01	15:01	04:01	15:01
16	03:01	09:01	03:01	09:01
17	04:04	12:01	04:04	12:01
18	09:01	15:01	09:01	15:01
19	07:01	15:01	07:01	15:01
20	01:01	15:02	01:01	15:02

Results are given in WHO HLA nomenclature: first two numbers indicate the main antigen, second two numbers (after the colon) indicate the allele specificity of this antigen. Additional expressed DBR alleles are separated by a slash.

Table 2. Functional results for multiplex real-time PCR-based HLA typing for 11 loci using hydrolysis probes in a plate format

Sample	Extraction	A*	A*	B*	B*	C*	C*	DRB1*	DRB1*	DRB345	DRB345	DQA1*	DQA1*	DQB1*	DQB1*	DPA1*	DPA1*	DPB1*	DPB1*
19	EZ2	02	03	07	57	06	07	07	15	4*01:03:01N	5*01	01	02	03:03	06	01	01	04:01	04:01
	EZ1	02	4	03	4	07	57	07	15	4*01:03:01N	5*01	01	02	03:03	06	01	01	04:01	04:01
22	EZ2	02	02	27	57	02	06	03:01	14	3*01	3*02	01	05	02	05	01	02	04:01	14:01
	EZ1	02	02	27	57	02	06	03:01	14	3*01	3*02	01	05	02	05	01	02	04:01	14:01
26	EZ2	01	24	18	47	06	07	15	15	5*01	5*01:01:08N	01	01	05	06	01	02	04:01	13:01
	EZ1	01	24	18	47	06	07	15	15	5*01	5*01:01:08N	01	01	05	06	01	02	04:01	13:01
27	EZ2	02	31	07	45	07	16	13	15	3*01	5*01	01	01	06	06	02	03	01:01	105:01
	EZ1	02	31	07	45	07	16	13	15	3*01	5*01	01	01	06	06	02	03	01:01	105:01
37	EZ2	02	11	15:13	55	01	08	04	12	3*03	4*01	03	06	03:01	04	01	02	02:01	05:01
	EZ1	02	11	15:13	55	01	08	04	12	3*03	4*01	03	06	03:01	04	01	02	02:01	05:01
38	EZ2	03	25	08	58	03:02	07	07	08	4*01	-	02	04	02	04	01	01	03:01	104:01
	EZ1	03	25	08	58	03:02	07	07	08	4*01	-	02	04	02	04	01	01	03:01	104:01
46	EZ2	24	26	35	44	04	05	08	13	3*01	-	01	04	04	06	01	01	04:02	04:02
	EZ1	24	26	35	44	04	05	08	13	3*01	-	01	04	04	06	01	01	04:02	04:02
64	EZ2	01	32	35	44	04	05	11	11	3*02	3*02	01	05	03:01	06	01	02	02:01	04:02
	EZ1	01	32	35	44	04	05	11	11	3*02	3*02	01	05	03:01	06	01	02	02:01	04:02
67	EZ2	02	03	15:10	35	03:03	04	12	14	3*02	3*02	01	05	03:01	05	01	01	04:01	04:01
	EZ1	02	03	15:10	35	03:03	04	12	14	3*02	3*02	01	05	03:01	05	01	01	04:01	04:01
71	EZ2	02	29	18	40:10	05	15	03:01	14	3*01	3*02	05	05	02	03:01	01	01	04:02	04:02
	EZ1	02	29	18	40:10	05	15	03:01	14	3*01	3*02	05	05	02	03:01	01	01	04:02	04:02

Results are given in WHO HLA nomenclature: column heading indicates the gene locus, * indicates molecular genetic testing. The numbers within a cell indicate the main antigen (first two numbers), the allele specificity of this antigen (second two numbers), silent mutations (third two numbers) and polymorphisms (fourth two numbers). N indicates zero allele (no expression); - indicates no additional DRB345 allele detected. EZ1: EZ1 Advanced XL; EZ2: EZ2 Connect MDx.

Table 3. Analytical method results for DNA concentration (ng/μL) and purity (ratio A_{260}/A_{280})

Sample ID	EZ1		EZ2		Sample ID	EZ1		EZ2		Sample ID	EZ1		EZ2	
	Concentration	Purity	Concentration	Purity		Concentration	Purity	Concentration	Purity		Concentration	Purity	Concentration	Purity
1	70.0	1.87	65.5	2.02	35	11.7	2.16	20.4	2.91	69	26.9	2.25	55.3	2.07
2	44.9	1.71	25.9	2.38	36	25.6	2.01	32.3	2.38	70	36.0	2.07	39.2	2.12
3	53.3	1.81	52.4	2.10	37	65.4	1.90	61.1	2.12	71	56.1	2.03	53.5	1.97
4	31.1	1.95	26.2	2.48	38	57.9	1.90	51.7	2.14	72	56.9	2.04	60.4	1.95
5	41.2	1.89	38.9	2.22	39	52.7	1.94	39.6	2.21	73	51.6	1.97	38.2	1.99
6	37.7	1.46	44.4	2.14	40	28.9	1.93	28.1	2.31	74	60.5	1.94	49.3	1.95
7	37.0	1.98	35.8	2.14	41	27.6	1.82	14.9	3.12	75	51.6	1.94	40.8	2.02
8	33.5	1.89	18.3	2.33	42	23.0	1.90	17.1	3.40	76	43.9	2.36	38.0	2.26
9	40.1	1.95	29.1	2.08	43	28.8	2.07	33.8	2.32	77	45.7	2.81	53.1	2.34
10	81.9	1.89	38.3	2.06	44	59.8	1.83	54.5	2.12	78	49.3	2.15	57.5	2.65
11	48.7	1.90	50.7	2.16	45	48.2	1.89	52.4	2.12	79	72.8	2.17	91.5	1.97
12	32.0	1.94	43.0	2.20	46	78.4	1.93	50.2	2.13	80	64.8	2.12	65.5	2.20
13	42.6	1.95	40.2	2.20	47	69.9	1.88	46.1	2.12	81	71.4	1.07	79.1	2.19
14	26.2	1.93	40.3	2.20	48	42.9	1.91	42.4	2.19	82	60.0	2.22	76.6	2.21
15	38.1	1.81	87.8	2.00	49	40.0	1.21	38.6	2.13	83	*	*	*	*
16	19.1	2.05	26.8	2.42	50	29.6	1.32	32.1	2.16	84	67.4	2.20	98.4	2.24
17	36.2	1.95	38.9	2.21	51	31.8	1.52	48.5	2.06	85	66.0	2.10	103.6	2.10
18	35.8	2.03	45.9	2.08	52	8.9	2.18	71.9	1.92	86	63.6	2.04	69.4	2.13
19	49.0	1.93	55.9	2.11	53	112.7	1.92	54.6	1.97	87	60.7	2.00	69.8	1.97
20	51.7	1.90	40.6	2.21	54	66.5	1.97	47.2	2.03	88	52.4	2.16	69.8	2.54
21	23.8	2.02	35.5	2.29	55	106.0	1.99	96.9	1.88	89	54.7	2.19	68.0	2.39
22	51.8	2.06	60.4	2.06	56	48.1	2.05	19.3	2.38	90	57.6	2.17	77.4	2.04
23	33.1	2.03	60.2	2.09	57	36.8	2.12	28.5	2.11	91	59.0	2.16	78.7	2.02
24	26.7	2.04	35.4	2.26	58	41.8	2.18	46.1	2.14	92	57.0	2.15	67.6	2.03
25	37.0	1.93	48.6	2.13	59	44.3	2.10	42.4	2.12	93	50.2	2.36	62.7	2.05
26	48.4	1.89	60.6	2.08	60	37.4	2.12	24.1	2.32	94	65.0	2.28	78.5	2.03
27	78.1	1.91	62.6	2.09	61	21.2	2.66	76.4	2.01	95	70.9	2.20	82.6	2.29
28	57.1	1.93	51.3	2.15	62	18.3	2.58	47.2	2.08	96	75.2	2.45	100.2	2.03
29	56.9	1.90	51.4	2.14	63	22.8	2.36	30.9	2.24	97	60.6	2.05	86.8	1.98
30	37.2	1.89	31.4	2.34	64	54.7	2.17	53.5	2.05	98	66.8	2.26	90.5	2.00
31	59.2	1.91	56.3	2.10	65	95.4	1.97	93.8	1.95	99	70.8	1.94	87.3	2.01
32	57.2	1.91	50.3	2.14	66	75.5	1.99	67.7	2.02	100	67.4	2.33	96.5	1.97
33	35.8	1.89	37.4	2.29	67	112.4	1.95	49.7	2.20	101	77.5	2.07	107.8	1.97
34	36.7	1.97	45.4	2.18	68	105.7	1.95	71.0	2.02	102	65.6	2.26	85.6	1.96

* Sample 83 was deleted as an outlier. EZ1: EZ1 Advanced XL; EZ2: EZ2 Connect MDx.

The results from multiplex real-time PCR-based HLA typing are listed in Table 4 and indicate the performance of the DNA extracted from both instruments was similar.

Real-time PCR results of the functional analysis obtained for the HLA typing are summarized in Table 2.

The mean concentration and purity of all samples were calculated and are presented in Table 3 and the means for the data according to instrument are presented in Table 4.

High variation of DNA concentration as well as purity was observed for the samples and is expected, as the

amount of DNA in the blood of individual donors varies. Notably, the variation was comparable between samples purified with both instruments.

DNA purity also varied, particularly with respect to high-purity values (above 2.0). This variation is likely related to OD measurement on the Nanodrop: blank measurement was performed with distilled water instead of the elution buffer (Buffer AVE) used in the EZ1 DSP DNA Blood Kit. This difference had the same impact on all measured eluates, and the DNA concentrations from samples purified with both instruments were comparable.

Table 4. Calculated mean concentration and mean purity of all purified DNA samples

	DNA concentration (ng/ μ L)			Mean purity (A_{260}/A_{280})		
	Mean	SD	Mean $\pm$ SD (EZ1 reference)	Concentration	Purity	Concentration
EZ1	51.2	20.7	30.5 – 71.9	2.01	0.24	1.77 – 2.25
EZ2	54.4	21.8	N/A	2.17	0.22	N/A

SD: standard deviation; N/A: not available. EZ1: EZ1 Advanced XL; EZ2: EZ2 Connect MDx.

Conclusions

This study confirms that the EZ2 Connect MDx can be used routinely in combination with the EZ1 DSP DNA Blood kit. The results of the HLA typing assays, SSO and

multiplex real-time PCR, indicate that the DNA extracted from whole blood by the EZ2 Connect MDx instrument performs well in downstream applications. Yields and purity obtained with the EZ2 Connect MDx were as good as or better than those obtained with the EZ1 Advanced XL.

Ordering Information

Product	Contents	Cat. no.
EZ2 Connect MDx	Compact benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 1-year warranty on parts and labor	9003230
EZ1 DSP DNA Blood Kit (48)	For 48 DNA preps: Prefilled Reagent Cartridges, Disposable Tip-Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes	62124



Learn more about how the EZ2 Connect MDx can power up your lab at www.qiagen.com/us/clp/ez2-connect-mdx

The EZ2 Connect MDx is intended for in vitro diagnostic use.

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