

Philisa[®] Thermal Cycler Application Note

Rapid PCR Amplification of Multiplex HLA Amplicons using a commercial SSO kit

Steve Kelly, Joel R. TerMaat, Ph.D., Streck, Inc.

Overview

The purpose of this study was to evaluate the use of the Philisa[®] Thermal Cycler to rapidly amplify HLA PCR products using a commercial SSO kit prior to hybridization and detection on a Luminex[®] 200[™] System. The amplification step currently requires up to 1.5 hours for completion.

The study results showed the Philisa Thermal Cycler reduced the total amplification time to less than 30 minutes with minimal alteration of the PCR protocol. All nine HLA loci under rapid cycling were concordant with traditional PCR samples, demonstrating that accurate SSO typing can be achieved while reducing run times by one hour.

Materials and Methods

PCR amplification of the LABType[®] SSO kit from Thermo Fisher Scientific for HLA amplification was optimized by Streck, Omaha, NE. The accuracy of the rapid PCR method was tested and verified by a clinical HLA lab in Maryland. The SpeedSTAR[™] HS DNA Polymerase from TaKaRa was utilized in the amplification mixture. Per reaction, the amplification mixture contained the following reagents:

Reaction Components		Amount (µl)	Modified Amplification Protocol		
			Step	Temperature and Incubation Time	# of cycles
D-Mix		13.8	Hot Start	96°C for 60 s	1
Amplification Primer Set		4.0	Cycle 1	96°C for 8 s	5
SpeedSTAR Taq		0.2		60°C for 14 s	
Patient DNA		2.0		72°C for 10 s	
Reaction total		20.0	Cycle 2	96°C for 6 s	35
				60°C for 12 s	
				72°C for 10 s	
			Final Extension	72°C for 3 min	1

The following HLA loci were tested using the modified Philisa protocol:

HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB345, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1

Results and Discussion

Figure 1 (see reverse side) illustrates that rapid cycling utilizing the Philisa Thermal Cycler had no adverse effect on the accuracy of the HLA testing procedure. Amplification time was reduced to 27 minutes, compared to 1 hour, 30 minutes for the standard cycling protocol. Additionally, an increase of mean fluorescent intensity and agarose gel band intensity indicate a more efficient amplification. Subsequent analysis on the Luminex 200 showed the nine HLA loci amplified with Philisa were in concordance with results using standard amplification techniques.

The ability of the Philisa Thermal Cycler to accurately perform rapid PCR amplification of HLA loci using commercial SSO kits has been demonstrated. This indicates the Philisa Thermal Cycler is compatible with Luminex technology and can be utilized for rapid tissue typing prior to organ transplantation.

See the reverse side of this application note for Figure 1.



800.843.0912
streck.com

Figure 1

Comparison of PCR amplification efficiency for a standard thermal cycler and the Philisa Thermal Cycler using Mean Fluorescence Intensity (MFI).

Sample ID	Exon 2 Positive Control (MFI)		Exon 3 Positive Control (MFI)		Negative		Typing Result	
	Standard	Philisa	Standard	Philisa	Standard	Philisa	Standard	Philisa
HLA-A								
1	3221	3887.51	1932.45	2159.34	10.82	12.75	23:PXVU 24:RAZT	23:PXVU 24:RAZT
2	3035.8	4029.91	2195.69	2380.13	10.57	13.5	02:PXTP 24:RAPK	02:PXTP 24:RAPK
HLA-B								
1	2492.61	3880.11	2893.18	4355.84	15.16	11.52	08:PZDG 44:JXRB	08:PZDG 44:JXRB
2	3712.61	3772.4	3068.18	4611.44	11.44	10.11	07:PYJP 51:PYTM	07:PYJP 51:PYTM
HLA-C								
1	4319.38	4757.85	2480.46	2653.57	8.73	13.21	04:RGHC 07:RFHF	04:RGHC 07:RFHF
2	4142.86	4927.13	2309.05	3085.98	9.49	12.33	01:RGFK 07:RGJG	01:RGFK 07:RGJG
HLA-DRB1								
1	3386.98	3774.72	NA	NA	8.38	11.44	03:RBMF 12:RAUX	03:RBMF 12:RAUX
2	3672.44	3393.91	NA	NA	10.42	10.27	09:RCWZ 11:RCXB	09:RCWZ 11:RCXB
HLA-DRB345								
1	4380.54	5083.52	NA	NA	10.17	9.86	DRB3*01:01 DRB3*02:CFXN	DRB3*01:01 DRB3*02:CFXN
2	4338.54	4933.4	NA	NA	11.49	10.1	DRB3*02:CFXN DRB4*01:MY	DRB3*02:CFXN DRB4*01:MY
HLA-DQA1								
1	2158.54	2287.78	2277.79	3371.22	10.82	11.25	05:01 05:BHS	05:01 05:BHS
2	2317.68	2332.97	3095.43	2832.09	12.5	9.47	03:BC 05:EF	03:BC 05:EF
HLA-DQB1								
1	3163.8	4532.43	1971.59	278.5	10.82	11.25	02:01 03:JVYH	02:01 03:JVYH
2	3372.81	3674.63	1967.77	2638.46	12.5	9.47	03:JVYH 03:RAUC	03:JVYH 03:RAUC
HLA-DPA1								
1	3821.34	3725.57	NA	NA	9.89	11.46	01:03 02:02	01:03 02:02
2	4134.57	3493.27	NA	NA	13.23	12.07	1:03	1:03
HLA-DPB1								
1	2332.4	2369.24	3266.77	3930.78	9.89	11.46	04:01 05:01	04:01 05:01
2	2481.28	2689.81	2550.07	3414.52	13.23	12.07	02:01 04:01	02:01 04:01