



Test-Durchführung / -Performance

Datum / Date

Unterschrift / Signature

Bemerkungen / Comments

Probe / Sample				
1	5	9	13	17
2	6	10	14	18
3	7	11	15	19
4	8	12	16	20
Thermocycler 1	Thermocycler 2	Thermocycler 3	Thermocycler 4	Thermocycler 5

Methodenablauf und Pipettierolumina der Einzelkomponenten für ein PCR-Reaktionsgefäß sind in allen **HLA-READY GENE** Kits gleich /
 Test procedure and pipetting volumina for one well are the same in all **HLA-READY GENE** kits

1.) Mastermix (MM) ansetzen, gut mischen / Pipette mastermix (MM), mix well

	Volume per well [µl]	Volume per typing (24+4* wells) [µl]	Multipliziert mit der Anzahl an Bestimmungen / multiplied by the number of typings	Endvolumen [µl]
Destilled Water	6	168	X	=
ReadyPCR	3	84	X	=
Taq-Polymerase (5U/µl)	0.08	2.2	X	=
Endvolumen MM	9.08	254	X	=

* 4 wells extra volume

Bei Mitführen einer **Negativkontrolle** (NK) 10 µl MM in das NK-Reaktionsgefäß (Tube Nr. 1) übertragen /
 When testing a **Negative Control** (NC) pipette 10 µl of MM into a NC reaction tube (tube no. 1)

2.) Aliquote 236 µl MM in 1,5 ml Tubes (je nach Anzahl der Bestimmungen / dependent on number of typings)

3.) Proben DNA zugeben, gut mischen / Add sample DNA, mix well

	Volume per well [µl]	Volume per typing (23+3* wells) [µl]
Mastermix (MM)	9.08	236
DNA (25-100 ng/µl)	1	26
Endvolumen MM-DNA	10.08	262

* 3 wells extra volume

4.) 10 µl MM-DNA pro Well (23x) in grüne PCR-Tubes verteilen / Dispense 10 µl MM-DNA per well (23x) in green PCR tubes

5.) RG mit Deckelstreifen dicht schließen und in Thermocycler übertragen /

Close wells tightly with tube caps and place the block into thermocycler

6.) PCR im Thermocycler starten (Programm für alle HLA-READY GENE Testkits gleich, s. Gebrauchsinformation) /

Start PCR in thermocycler (same Program for all HLA-READY GENE kits, see user manual)



 Name / Name

 Vorname / First name

 Geburtsdatum / Date of birth

 Proben-Nr. / Sample I.D.

 Herkunft / Origin

 Diagnose / Diagnosis

 Test-Durchführung / -Performance

 Datum / Date

 Unterschrift / Signature

 Test-Überprüfung / -Review

 Datum / Date

 Unterschrift / Signature

 Bemerkungen / Comments

Ergebnis / Result

	H	G	F	E	D	C	B	A			
HLA-A*	1	2	3	4	5*	6	7	8			
1	Negative Control	01..	02..	03.. /	0112 /	0217 / 2301,03-12 /	2302 /	23.. / 24.. /			
5				3204 / 3602	11.. /	2413,18,24 / 2907	24..	3108 / 3205			
9		<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="2419,44 / 2504 / 2608"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>		
	<input type="text" value="430 bp NC"/>	<input type="text" value="80 bp"/>	<input type="text" value="135 bp"/>	<input type="text" value="230 bp"/>	<input type="text" value="480 bp"/>	<input type="text" value="210 bp"/>	<input type="text" value="210 bp"/>	<input type="text" value="95 bp"/>			
	9	10	11	12*	13*	14	15	16			
2	1110 / 25.. / 26.. /	25..	26..	0113 / 0234,... / 03..	1111 / 4301	0113 / 11.. / 2419 /	29..	30..			
6	34.. / 66.. /				25.. / 26.. /	6602,03			2502 / 2613,19 /	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>
10	68.. / 69..			<input type="text" value="1070 bp IC"/>	<input type="text" value="800 bp IC"/>	4301 / 6601,04			34.. / 6601,04	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>
	<input type="text" value="800 bp IC"/>	<input type="text" value="475 bp"/>	<input type="text" value="75 bp"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>			
	<input type="text" value="200 bp"/>			<input type="text" value="225 bp"/>	<input type="text" value="200 bp"/>	<input type="text" value="95 bp"/>	<input type="text" value="200 bp"/>	<input type="text" value="90 bp"/>			
				<input type="text" value="170 bp"/>	<input type="text" value="170 bp"/>						
	17*	18	19	20	21	22	23	24*			
3	31..	2503 /	33..	74..	0210,... / 1116 /	0255 / 2622 /	01.. / 36..	80..			
7		32..				68..			69..	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>
11		<input type="text" value="800 bp IC"/>			<input type="text" value="1070 bp IC"/>	<input type="text" value="800 bp IC"/>			<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>	<input type="text" value="800 bp IC"/>
	<input type="text" value="260 bp"/>	<input type="text" value="260 bp"/>	<input type="text" value="200 bp"/>	<input type="text" value="155 bp"/>	<input type="text" value="140 bp"/>	<input type="text" value="375 bp"/>	<input type="text" value="170 bp"/>	<input type="text" value="800 bp IC"/>			
	<input type="text" value="180 bp"/>							<input type="text" value="155 bp"/>			
								<input type="text" value="70, 75 bp"/>			

¹ einige seltenere Allele ausgenommen (vollständige Liste: Interpretation -/Specificity Table) / some rare alleles excluded (complete list: Interpretation -/Specificity Table)
Fettdruck: Allel(gruppen) mit Häufigkeiten von mind. 0,001 / **Bold letters:** parts of allele groups or alleles with min. frequency of 0,001 (Ref.: www.allelefreqencies.net)
IC: PCR Produkte der internen Kontrollprimermixe / PCR products of the internal control primer mixes

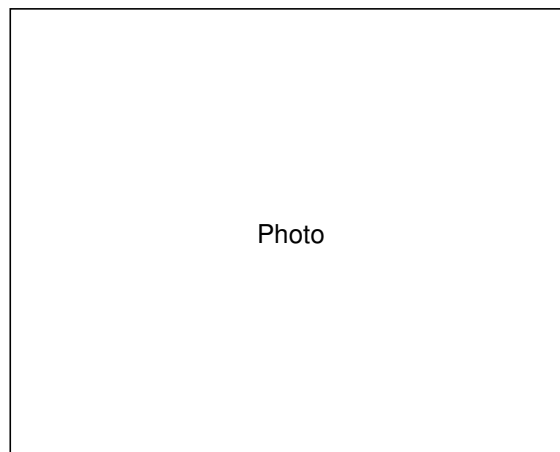
Mix # 5: kreuzreaktiv mit / crossreactive to Cw*0414

Mix # 12 (multiplex): 225 bp: A*0234,35,56,62,*0301-07,09-11N,13,14; 170 bp: A*2501-04,*2601-03,05,07,08,10-23,*4301,*6601,04

Mix # 13 (multiplex): 200 bp: A*1111,*4301; 170 bp: A*6602,03

Mix # 17 (multiplex): 260 bp: A*3107,08; 180 bp: A*3101-07,09-12

Mix # 24 (multiplex): 155 bp: A*8001; 70/ 75 bp: A*0255,*2603,05,06,*6805,15,20



HLA-READY GENE A Low

SSP HLA-A low resolution

Spezifitätstabelle / Specificity Table

IN VITRO DIAGNOSTICUM



LOT

G950085



2006-12

Allele list based on release
2.9.0 (Apr 2005) of the
IMGT/HLA Sequence Database

Tube No.		Amplified HLA-A* alleles	Size of PCR Product [bp]	Size of IC [bp]	5' primer(s) ¹	3' primer(s) ¹
1	1H	Negative Control	430	-		
2	1G	0101-09,11N,13	80	800	527 TGC	570 CCG
3	1F	020101-020108,0110,0111,0202-41,43N-47,49,51-54,56-60,62-78	135	800	144 GCA	240 GGA
4	1E	0301-04,07-09,11N-16 / 3204 / 3602	230	800	363 ATA	555 CCA
5 ²	1D	0112 / 1101-07,09-11,13-17,19-22 / 2419,44 / 2504 / 2608	480	800	341 GGA	538 CTG
6	1C	0217 / 2301,03-12 / 2406,13,18,22,24 / 2907	210	800	368 GTT	538 CAA
7	1B	2402-11N,17,19-23,25-50 / 2302	210	800	368 GTT	538 CCA, 539 TCT
8	1A	2301-08N,10-12 / 2402-03,05-07,09N-18,20-23,25-27,30,32-41,43,45N-51 / 3108 / 3205	95	800	265 CAG	317 GGA
9	2H	1110 / 2501,02,04 / 2601-06,08-15,17,18,21-24 / 3401-06 / 6601-04 / 6801-09,11N,12,15-28 / 6901	200	800	98 CTA	259 GTT
10	2G	2501-04	475	1070	317 GCT	517 CGT
11	2F	2601,02,04,07-18,20,22-24	75	800	261 AAC, 257 CGG	299 TCG
12	2E	0113 / 0234,35,56,62 / 0301-07,09-11N,13-16 2501-04 / 2601-03,05,07,08,10-24 / 4301 / 6601,04	225 170	800	98 CTT 423 GCT	282 GAC 559 CCG
13	2D	1111 / 4301 6602,03	200 170	800	98 CTA 423 GCT	257 GCA 559 CTC
14	2C	0113 / 1101-11,13-16,20-23 / 2419 / 2502 / 2613,19 / 3401-06 / 6601,04	95	800	282 CAG	341 CGT
15	2B	2901-13	200	800	98 CAC	257 GCA
16	2A	3001-15	90	800	363 ATA	411 TCA
17	3H	3101-07,09-12 3107,08	180 260	800	97 TCA	238 CCT 317 GGA
18	3G	2503 / 3201-06,08,09	260	800	98 CTT	317 GGA
19	3F	3301-07	200	1070	98 CAC	259 GTT
20	3E	7401-09	155	800	180 TTT	299 CCA, 299 CCA
21	3D	0210,50,52,73 / 1116 / 6801-11N,13-28	140	800	391 ACG, 391 ACG	497 TGG
22	3C	0255 / 2622 / 6901	375	800	261 AAC	355 GAC
23	3B	0101-06,08-13 / 3601-04	170	800	203 GAA	341 CGT
24	3A	8001 0255 / 2603,05,06,21 / 6805,15,20	155 70, 75	800	176 GCA 261 AAC	292 GTT 292 GTG, 299 TCT

Fettdruck: Allel(gruppen) mit Häufigkeiten von mind. 0,001 / **Bold letters:** parts of allele groups or alleles with min. frequency of 0,001 (Ref.: www.allelefreqencies.net)

IC: PCR Produkte der internen Kontrollprimermixe / PCR products of the internal control primer mixes

¹ Nukleotidposition und Codon der 3' Primerenden / nucleotide position and codon of primers 3' ends

² **Mix # 5:** kreuzreaktiv mit Cw*0414 / crossreactive to Cw*0414



Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A	3H	3G	3F	3E	3D	3C	3B	3A	
Size of PCR Product [bp]	-	80	135	230	480	210	210	95	200	475	75	225 170	200 170	95	200	90	260 180	260	200	155	140	375	170	155 75	
A Allele Specificity																									
Negative Control	1																								
A*0101-06,08,09,11N		2																					23		
A*0107		2																							
A*0112					5																		23		
A*0113		2										12L		14									23		
A*0201-09,11-16,18-33,36-41,43-47,49,51,53N,54,57-60,63-72,74-78			3																						
A*020109,42,48,61																									
A*0210,52,73			3																		21				
A*0217			3			6																			
A*0234,35,56,62			3									12L													
A*0250 / 6810,13,14																					21				
A*0255																						22		24s	
A*0301-04,07,09,11N,13-16				4								12L													
A*0305,06,10 / 023502												12L													
A*0308,12				4																					
A*1101-07,09,13-15,20-22					5									14											
A*1108,23														14											
A*1110					5			9						14											
A*1111					5								13L	14											
A*1112,18																									
A*1116					5									14							21				
A*1117,19					5																				
A*2301,03-08N,10-12 / 2413,18						6		8																	
A*2309 / 2424						6																			
A*2402,03,05-07,09-11N,17,20-23,25-27,30,32-41,43,45N-50 / 2302							7	8																	
A*2404,08,28,29,31,42							7																		
A*2414,15,51								8																	
A*2419					5		7							14											
A*2444					5		7																		
A*2501									9	10		12s													
A*2502									9	10		12s		14											
A*2503										10		12s						18							
A*2504					5				9	10		12s													
A*2601,02,10-12,14,15,17,18,23,24									9		11	12s													
A*2603,05,21									9			12s											24s		
A*2604,09									9		11														
A*2606									9														24s		
A*2607,16,20											11	12s													
A*2608					5				9		11	12s													
A*2613									9		11	12s		14											
A*2619												12s		14											
A*2622									9		11	12s										22			
A*2901-06,08N,09-13																	15								
A*2907																	15								
A*3001-15						6												16							
A*3101-06,09-12																		17s							
A*3107																		17sL							
A*3108								8										17L							
A*3201-03,06,08,09																					18				
A*3204				4																	18				
A*3205								8													18				
A*3207																									
A*3301-07																					19				
A*3401-06									9					14											
A*3601,03,04 / 0110																							23		
A*3602				4																			23		
A*4301												12s13L													
A*6601,04									9			12s		14											
A*6602,03									9			13s													
A*6801-04,06-09,11N,16-19,21-28									9												21				
A*6805,15,20									9												21		24s		
A*6812									9																
A*6901									9													22			
A*7401-09																					20				
A*7410																									
A*8001																								24L	

gelegentlich schwache Bande / occasionally weak band

- Multiplex Mix !** 12 s L 2 spezifische Banden / 2 specific bands: s(mall) 170 bp, L(arge) 225 bp
- Multiplex Mix !** 13 s L 2 spezifische Banden / 2 specific bands: s(mall) 170 bp, L(arge) 200 bp
- Multiplex Mix !** 17 s L 2 spezifische Banden / 2 specific bands: s(mall) 180 bp, L(arge) 260 bp
- Multiplex Mix !** 24 s L 2 spezifische Banden / 2 specific bands: s(mall) 70/ 75 bp, L(arge) 155 bp

SSP HLA-A low resolution
Frequency Table



Frequent alleles:

Allele Specificity	Freq ¹	Ser. Type
A*010101	c*	A1
A*0102	ai	A1
A*0106	ie	-
A*02010101	c*	A2
A*0202	c*	A2
A*0203	o,ie	A203
A*0204	c*	A2
A*0205	c*	A2
A*0206	c*	A2
A*0207	c*	A2
A*0208	a	A2
A*0210	o	A210
A*0211	c*	A2
A*0214	a	A2
A*021701	c*	A2
A*0219	ai	-
A*0222	c*	A2
A*0224	c*	A2
A*0226	c*	-
A*0236	ie	-
A*03010101	c*	A3
A*030102	ie	A3
A*0302	c*	A3
A*110101	c*	A11
A*1102	c*	A11
A*1104	ai	A11
A*2301	c*	A23(9)
A*24020101	c*	A24(9)
A*24020102L	c*	Low A24
A*240301	c*	A2403
A*2404	h	A24(9)
A*2405	ie	A24(9)
A*2406	ie	A24(9)
A*2407	o,ie	A24(9)
A*2408	o,ie	A24(9)
A*2409N	c*	Null
A*2410	o	A9
A*2423	x	A24(9)
A*2424	ie	-

Allele Specificity	Freq ¹	Ser. Type
A*250101	c*	A25(10)
A*2502	c*	A10
A*2601	c*	A26(10)
A*2602	c*	A26(10)
A*2605	c*	A26(10)
A*260701	c*	A26(10)
A*2608	c*	A26(10)
A*2611N	ie	Null
A*2614	ie	-
A*29010101	c*	A29(19)
A*290201	c*	A29(19)
A*3001	c*	A30(19)
A*3002	c*	A30(19)
A*3004	c*	A30(19)
A*310102	c*	A31(19)
A*3108	ie	-
A*3201	c*	A32(19)
A*3202	c*	A32(19)
A*3204	ie	-
A*3301	c*	A33(19)
A*330301	c*	A33(19)
A*3305	c*	A33(19)
A*3401	c*	A34(10)
A*3402	c*	A34(10)
A*3601	c*	A36
A*4301	b	A43
A*6601	c*	A66(10)
A*6602	c*	A66(10)
A*680101	c*	A68(28)
A*680102	c*	A68(28)
A*6802	c*	A68(28)
A*680301	c*	A28
A*6805	m	A68(28)
A*6901	c*	A69(28)
A*7401	o	A74(19)
A*7403	ai	A74(19)
A*8001	c*	A80

¹ allele frequency min. 0,001 (Ref.: www.allelefrequencies.net): a arab, ai amerindian, b black, c* caucasoid (and other)
h hispanic, ie indo-european, m mixed (Mexico Mestizos), o oriental, x Yupik Natives