

101.122-24/06 – including *Taq* pol., IFU-01
101.122-24u/06u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **48X**

Lot-specific information
Olerup SSP® DRB4

Product number:	101.122-24/06 – including <i>Taq</i> pol. 101.122-24/06u – without <i>Taq</i> pol.
Lot number:	48X
Expiry date:	2017-April-01
Number of tests:	24 test – Product No. 101.122-24 6 tests – Product No. 101.122-06
Number of wells per test:	13+1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 48X.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® DRB4 LOT (67S)

A well containing Negative Control primer pairs has been added.

The format of the Product Insert and Worksheet have been changed.

The DRB4 primer set, specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP®* DRB4 lot was made (Lot No. 67S). The kit design is based on IMGT/HLA database 3.17.0.

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
8	-	-	Strength of control band has been optimized.
14	-	-	Negative Control.

Change in revision R01 compared to R00:

1. Primer mix 8 may give rise to a lower yield of HLA-specific PCR product than the other DRB4 primer mixes. A foot note has been added in the Specificity Table.

Change in revision R02 compared to R01:

1. The genotype of the 9025/DEU cell line is DRB4*01:03. This has been corrected in the Cell Line Validation Sheet.

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Well **14** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP®* HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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PRODUCT DESCRIPTION

DRB4 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB4*01:01:01:01 to DRB4*03:01N alleles.

PLATE LAYOUT

Each test consists of 14 PCR reactions in a 16 well cut PCR plate. Wells 15 to 16 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	NC	empty	empty

The 16 well cut PCR plate is marked with 'DRB4' in silver/gray ink.

Well No. 1 is marked with the Lot No. '48X'.

Wells 1 to 14 – DRB4 high resolution primers.

Well 14 – Negative Control (NC).

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 16 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only DRB4 alleles will be amplified by the primers in the DRB4 SSP subtyping kit¹. Thus, the interpretation of DRB4 SSP subtypings is not influenced by alleles of other DRB genes.

For further details see Specificity Table.

¹The DRB1*15:01:01:01-15:112 and the DRB1*16:01:01-16:05:02, 16:07-16:24 alleles might be faintly amplified by primer mix 7.

UNIQUELY IDENTIFIED ALLELES

All the DRB4 alleles, i.e. **DRB4*01:01:01:01 to DRB4*03:01N**, recognized by the HLA Nomenclature Committee in July 2014^{1,2} will give rise to unique amplification patterns by the primers in the DRB4 subtyping kit.

The DRB4 subtyping kit cannot distinguish the silent mutation in the DRB4*01:03:01:01, 01:03:01:03-01:03:04 alleles.

¹DRB4 alleles listed on the IMGT/HLA web page 2014-July-25, release 3.17.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB4 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

DRB4 SSP subtyping

Specificities and sizes of the PCR products of the 13+1 primer mixes used for DRB4 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB4 ³ alleles
1	185 bp	515 bp	*01:01:01:01, 01:03:01:01-01:03:04, 01:05-01:08
2⁶	140 bp	430 bp	*01:02
3	130 bp	430 bp	*01:01:01:01, 01:04 ² -01:05 ² , 01:06, 01:07 ² -01:08 ² , 02:01N, 03:01N
4	245 bp	515 bp	*01:01:01:01-01:03:01:01, 01:03:01:03-01:04, 01:05 ² , 01:06-01:08, 02:01N
5⁵	155 bp	430 bp	*01:03:01:02N
6	190 bp	430 bp	*01:04
7⁷	155 bp	430 bp	*01:02-01:03:04, 01:04 ² -01:05 ² , 01:07 ² -01:08 ²
8⁹	290 bp	515 bp	*01:01:01:01, 01:04 ² -01:08 ² , 02:01N ² , 03:01N
9⁶	155 bp	515 bp	*01:05
10⁴	85 bp	515 bp	*02:01N
11⁴	110 bp	430 bp	*01:06
12	210 bp	430 bp	*01:07
13	215 bp	430 bp	*01:08
14⁸	Negative Control		

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DRB4 SSP typings.

When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases. In the presence of a specific amplification the intensity of the control band

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often decreases.

³For several DRB1 alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

The complete 2nd exon nucleotide sequence of the DRB4*01:05 allele is not known. Thus, it is not known whether the DRB4*01:05 allele will be amplified by primer mix 4 or not.

⁴HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁵Primer mix 5 may have tendencies of unspecific amplifications.

⁶Primer mixes 2 and 9 have a tendency to giving rise to primer oligomer formation.

⁷The DRB1*15:01:01:01-15:112 and the DRB1*16:01:01-16:05:02, 16:07-16:24 alleles might be faintly amplified by primer mix 7.

⁸Primer mix 14 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

⁹Primer mix 8 may give rise to a lower yield of HLA-specific PCR product than the other DRB4 primer mixes.

‘?’, nucleotide sequence information not available for the primer matching sequence.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Length of spec. PCR product	185	140	130	245	155	190	155	290	155	85	110	210	215
Length of int. pos. control ¹	515	430	430	515	430	430	430	515	515	515	430	430	430
5'-primer(s) ²	28(170) 5'-gAT 3'	42(213) 5'-AgT 3'	105(401) 5'-AAA 3'	1 st I 5'-ggg 3'	1 st I 5'-CAA 3'	28(170) 5'-gAT 3'	96(375) 5'-CAA 3'	2 nd I 5'-TgA 3'	42(213) 5'-AgT 3'	28(170) 5'-gAT 3'	111(421) 5'-ACT 3'	28(170) 5'-gAT 3'	28(170) 5'-gAT 3'
3'-primer(s) ³	76(314) 5'-TgT 3'	76(314) 5'-TgC 3'	134(490) 5'-gCT 3'	1 st I 5'-TgC 3'	42(213) 5'-TCA 3'	77(317) 5'-AgT 3'	134(490) 5'-gCC 3'	2 nd I 5'-TTC 3'	80(328) 5'-gTg 3'	42(213) 5'-TCA 3'	134(490) 5'-gCT 3'	83(337) 5'-CCg 3'	86(346) 5'-CTT 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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CELL LINE VALIDATION SHEET												
DRB4 SSP kit ²												
				Well								
				1	2	3	4	5	6	7	8	9
				10	11	12	13	14	15	16	17	18
				201202701	201443202	201202703	201202704	201202705	201202706	201202707	201443208	201443209
				201202710	201202711	201202712	201202713	201202714	201202715	201202716	201202717	201202718
				201202719	201202720	201202721	201202722	201202723	201202724	201202725	201202726	201202727
				201202728	201202729	201202730	201202731	201202732	201202733	201202734	201202735	201202736
				201202737	201202738	201202739	201202740	201202741	201202742	201202743	201202744	201202745
				201202746	201202747	201202748	201202749	201202750	201202751	201202752	201202753	201202754
				201202755	201202756	201202757	201202758	201202759	201202760	201202761	201202762	201202763
				201202764	201202765	201202766	201202767	201202768	201202769	201202770	201202771	201202772
				201202773	201202774	201202775	201202776	201202777	201202778	201202779	201202780	201202781
				201202782	201202783	201202784	201202785	201202786	201202787	201202788	201202789	201202790
				201202791	201202792	201202793	201202794	201202795	201202796	201202797	201202798	201202799
				201202800	201202801	201202802	201202803	201202804	201202805	201202806	201202807	201202808
				201202809	201202810	201202811	201202812	201202813	201202814	201202815	201202816	201202817
				201202818	201202819	201202820	201202821	201202822	201202823	201202824	201202825	201202826
				201202827	201202828	201202829	201202830	201202831	201202832	201202833	201202834	201202835
				201202836	201202837	201202838	201202839	201202840	201202841	201202842	201202843	201202844
				201202845	201202846	201202847	201202848	201202849	201202850	201202851	201202852	201202853
				201202854	201202855	201202856	201202857	201202858	201202859	201202860	201202861	201202862
				201202863	201202864	201202865	201202866	201202867	201202868	201202869	201202870	201202871
				201202872	201202873	201202874	201202875	201202876	201202877	201202878	201202879	201202880
				201202881	201202882	201202883	201202884	201202885	201202886	201202887	201202888	201202889
				201202890	201202891	201202892	201202893	201202894	201202895	201202896	201202897	201202898
				201202899	201202900	201202901	201202902	201202903	201202904	201202905	201202906	201202907
				201202908	201202909	201202910	201202911	201202912	201202913	201202914	201202915	201202916
				201202917	201202918	201202919	201202920	201202921	201202922	201202923	201202924	201202925
				201202926	201202927	201202928	201202929	201202930	201202931	201202932	201202933	201202934
				201202935	201202936	201202937	201202938	201202939	201202940	201202941	201202942	201202943
				201202944	201202945	201202946	201202947	201202948	201202949	201202950	201202951	201202952
				201202953	201202954	201202955	201202956	201202957	201202958	201202959	201202960	201202961
				201202962	201202963	201202964	201202965	201202966	201202967	201202968	201202969	201202970
				201202971	201202972	201202973	201202974	201202975	201202976	201202977	201202978	201202979
				201202980	201202981	201202982	201202983	201202984	201202985	201202986	201202987	201202988
				201202989	201202990	201202991	201202992	201202993	201202994	201202995	201202996	201202997
				201202998	201202999	201203000	201203001	201203002	201203003	201203004	201203005	201203006
				201203007	201203008	201203009	201203010	201203011	201203012	201203013	201203014	201203015
				201203016	201203017	201203018	201203019	201203020	201203021	201203022	201203023	201203024
				201203025	201203026	201203027	201203028	201203029	201203030	201203031	201203032	201203033
				201203034	201203035	201203036	201203037	201203038	201203039	201203040	201203041	201203042
				201203043	201203044	201203045	201203046	201203047	201203048	201203049	201203050	201203051
				201203052	201203053	201203054	201203055	201203056	201203057	201203058	201203059	201203060
				201203061	201203062	201203063	201203064	201203065	201203066	201203067	201203068	201203069
				201203070	201203071	201203072	201203073	201203074	201203075	201203076	201203077	201203078
				201203079	201203080	201203081	201203082	201203083	201203084	201203085	201203086	201203087
				201203088	201203089	201203090	201203091	201203092	201203093	201203094	201203095	201203096
				201203097	201203098	201203099	201203100	201203101	201203102	201203103	201203104	201203105
				201203106	201203107	201203108	201203109	201203110	201203111	201203112	201203113	201203114
				201203115	201203116	201203117	201203118	201203119	201203120	201203121	201203122	201203123
				201203124	201203125	201203126	201203127	201203128	201203129	201203130	201203131	201203132
				201203133	201203134	201203135	201203136	201203137	201203138	201203139	201203140	201203141
				201203142	201203143	201203144	201203145	201203146	201203147	201203148	201203149	201203150
				201203151	201203152	201203153	201203154	201203155	201203156	201203157	201203158	201203159
				201203160	201203161	201203162	201203163	201203164	201203165	201203166	201203167	201203168
				201203169	201203170	201203171	201203172	201203173	201203174	201203175	201203176	201203177
				201203178	201203179	201203180	201203181	201203182	201203183	201203184	201203185	201203186
				201203187	201203188	201203189	201203190	201203191	201203192	201203193	201203194	201203195
				201203196	201203197	201203198	201203199	201203200	201203201	201203202	201203203	201203204
				201203205	201203206	201203207	201203208	201203209	201203210	201203211	201203212	201203213
				201203214	201203215	201203216	201203217	201203218	201203219	201203220	201203221	201203222
				201203223	201203224	201203225	201203226	201203227	201203228	201203229	201203230	201203231
				201203232	201203233	201203234	201203235	201203236	201203237	201203238	201203239	201203240
				201203241	201203242	201203243	201203244	201203245	201203246	201203247	201203248	201203249
				201203250	201203251	201203252	201203253	201203254	201203255	201203256	201203257	201203258
				201203259	201203260	201203261	201203262	201203263	201203264	201203265	201203266	201203267
				201203268	201203269	201203270	201203271	201203272	201203273	201203274	201203275	201203276
				201203277	201203278	201203279	201203280	201203281	201203282	201203283	201203284	201203285
				201203286	201203287	201203288	201203289	201203290	201203291	201203292	201203293	201203294
				201203295	201203296	201203297	201203298	201203299	201203300	201203301	201203302	201203303

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¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

³The DRB1*15:01:01:01-15:112 and the DRB1*16:01:01-16:05:02, 16:07-16:24 alleles might be faintly amplified by primer mix 7.

No DNAs carrying the alleles to be amplified by primer solutions 6 and 9 to 13 were available. The specificities of the primers in primer solutions 6 and 9 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer. In primer solutions 10 and 11 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 12 and 13 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. One additional 3'-primer in primer solution 1 was tested by adding one additional 5'-primer.

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Lot-specific information

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