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Lot No.: 18R Lot-specific information

Olerup SSP® HLA-B*59

Product number: 101.554-06 – including *Taq* pol.

101.554-06u - without Tag pol.

Lot number: 18R

Expiry date: 2015-March-01

Number of tests: 6 Number of wells per test: 8

Storage - pre-aliquoted primers: dark at -20°C

- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. 18R.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® HLA-B*59 LOT (95K).

The HLA-B*59 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

The Lot-specific information for HLA-B*59 including and without *Taq* polymerase is now described in one common Product Insert.

The HLA-B*59 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP[®] HLA-B*59 lot **(Lot No. 95K)**.

The HLA-B*59 primer set is unchanged compared to the previous lot.

¹As described in section Uniquely Identified Alleles.

Lot No.: 18R Lot-specific information

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PRODUCT DESCRIPTION

HLA-B*59 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*59:01 to B*59:05 alleles.

PLATE LAYOUT

Each HLA-B*59 test consists of 8 PCR reactions in an 8 well cut PCR plate.

	1	2	3	4	5	6	7	8
--	---	---	---	---	---	---	---	---

The 8 well PCR plate is marked with 'B59' in silver/gray ink.

Well No. 1 is marked with the Lot No. '18R'.

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 heat-sealed with a PCR-compatible foil.

Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-B*59 SSP subtypings will be influenced by three B*07, two B*08, several B*13, four B*15, two B*27, two B*35, eight B*39, several B*40, eleven B*41, the B*42:04, seven B*44, most B*45, two B*46, most B*49, the B*50, most B*51, the B*52, the B*54, the B*55, the B*56, two B*58, the B*73, the B*78, three B*81, the B*82 and the B*83:01 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*59, i.e. **B*59:01 to B*59:05**, recognized by the HLA Nomenclature Committee in July 2012¹ will be amplified by the primers in the HLA-B*59 SSP kit.

The HLA-B*59 kit enables separation of the confirmed HLA-B*59 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA-B*59 alleles is listed below.

The HLA-B*59 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles

¹HLA-B alleles listed on the IMGT/HLA web page 2012-July-12, release 3.9.0, www.ebi.ac.uk/imgt/hla

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ALLELE CONFIRMATION STATUS

Allele	Status ¹
B*59:01:01:01	Confirmed
B*59:01:01:02	Unconfirmed
B*59:02	Unconfirmed
B*59:03	Unconfirmed
B*59:04	Confirmed
B*59:05	Unconfirmed

¹Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2012-July-12, release 3.9.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 5 alleles generate 5 amplification patterns that can be combined in 15 homozygous and heterozygous combinations. 4 of these genotypes do not give rise to unique amplification patterns.

```
*59:01, *59:04 = *59:04, *59:04
++----+ *59:01, *59:05 = *59:05, *59:05
```

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Lot No.: 18R Lot-specific information

SPECIFICITY TABLE

HLA-B*59 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-B*59 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*59 alleles ³	Other amplified HLA-B alleles ⁴
1	430 bp	800 bp	*59:01:01:01- 59:05	*54:12, 56:21
2 ⁵	105 bp	1070 bp	*59:01:01:01- 59:01:01:02, 59:04-59:05	*07:78, 13:02:01-13:03, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:34, 13:37-13:38, 13:40-13:42, 13:44-13:45, 13:48-13:49N, 13:53-13:56N, 13:58, 15:42, 35:60, 44:15, 44:18, 45:01, 45:03-45:08, 45:10-45:14, 46:11, 46:18, 49:01:01-49:03, 49:06-49:17, 49:19N-49:22, 50:01:01-50:02, 50:04-50:08, 50:10-50:11, 50:13, 50:15, 51:15, 52:25, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:16-54:27, 55:01:01-55:01:08-55:03, 55:05, 55:07, 55:09-55:12, 55:15-55:16, 55:18-55:19, 55:21-55:22, 55:24-55:26, 55:29-55:31, 55:33-55:38, 55:40-55:41, 55:43, 55:45-55:48, 55:50, 55:52, 55:54-55:56, 56:01:01-56:01:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:30, 56:33-56:36
3 ⁵	105 bp	1070 bp	*59:02	*07:84, 08:09, 08:84, 13:35, 15:83, 27:14, 27:81, 40:06:01:01-40:06:05, 40:44, 40:53, 40:70, 40:75, 40:83, 40:93, 40:95-40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:161-40:162, 40:165, 40:167, 40:177, 40:190, 41:01, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 41:20-41:21, 42:04, 44:20, 44:47, 44:100, 49:18, 50:14, 51:01:01-51:03, 51:05, 51:07:01-51:12, 51:14, 51:16-51:24:04, 51:26-51:34,

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Lot No.: 18R Lot-specific information

			•	
4	150 bp	1070 bp		51:36, 51:38-51:41N, 51:43-51:44N, 51:48-51:55, 51:57-51:58, 51:60-51:61:02, 51:65-51:80, 51:82-51:91, 51:93-51:96, 51:98N, 51:100-51:105, 51:107-51:130, 51:132, 51:134, 51:136-51:137, 52:01:01:01-52:13, 52:15-52:24, 52:26-52:30, 54:04, 54:11, 54:15, 55:17, 55:20, 55:27-55:28, 56:05:01-56:06, 56:15, 56:21, 58:08:01-58:08:02, 78:01:01-78:07 *54:12
5	400 bp	1070 bp		*07:65, 35:76, 40:166 [?] , 44:90, 54:01:01-54:06, 54:08N-54:23, 54:25-54:27, 55:01:01-55:05, 55:07-55:17, 55:19-55:33, 55:35-55:46, 55:48-55:56, 56:01:01-56:13, 56:15-56:16, 56:18-56:22, 56:24-56:32, 56:34-56:36, 81:01, 81:03 [?] -81:04N [?] , 82:01-82:03, 83:01
6 ⁵	105 bp	1070 bp	*59:03	*13:46, 15:137, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 40:86, 45:02, 50:09, 51:13:01-51:13:02, 51:92, 52:14, 55:13, 56:22, 73:01-73:02
7	180 bp	1070 bp	*59:04	*13:03, 13:48, 15:73, 40:71, 44:10, 45:14, 46:11, 51:15, 51:62, 51:106, 52:25, 54:03, 56:01:01-56:02, 56:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:20:01-56:20:02, 56:24-56:30, 56:33-56:36, 82:01-82:03
8	235 bp	1070 bp	*59:05	

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 HLA-B*59 SSP typings.

When the primers in a primer mix can give rise to 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 respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

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 inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low



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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 two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp internal positive control band in order to help in the correct orientation.

In the presence of a specific amplification the intensity of the control band often decreases.

³For several HLA-B alleles 1st exon 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. We assume that unknown sequences in these regions are conserved within allelic groups.

⁴Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*59 alleles will be amplified by primer mixes 1 to 7.

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

'?', nucleotide seguence information for the primer matching region not known.

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INTERPRETATION TABLE								
HLA-B*	59 SS	P suk	typin	q				
Amplification patter				_	lleles			
	Well							
	1	2	3	4	5	6	7	8
Length of spec.	430	105	105	150	400	105	180	235
PCR product								
Length of int.	800	1070	1070	1070	1070	1070	1070	1070
pos. control ¹								
5'-primer ²	48	357	357	206	41	357	420	357
o printer	_		^{5'} -Tgg ^{3'}					
	3	- 33	- 33	33		- 33		- 33
3'-primer ³	309	420	419	317	272	419	559	553
	5' -ATC 3'	^{5'} -gCT ^{3'}	^{5'} -CgT ^{3'}	^{5'} -ggA ^{3'}	^{5'} -TgT ^{3'}	^{5'} -CgA ^{3'}	^{5'} -CAg ^{3'}	^{5'} -CTg ^{3'}
Well No.	1	2	3	4	5	6	7	8
HLA-B allele ⁴								
*59:01:01:01- 59:01:01:02	1	2						
*59:02	1		3					
*59:03	1					6		
*59:04	1	2					7	
*59:05	1	2						8
*07:65, 35:76, 44:90, 54:06, 54:09, 54:14, 55:01:04, 55:01:07, 55:04, 55:08, 55:14, 55:23, 55:32, 55:39, 55:42, 55:44, 55:49, 55:51, 55:53, 56:03, 56:09-56:12, 56:18, 56:31-56:32, 81:01, 83:01					5			
*07:78, 13:02:01-13:02:13, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:34, 13:37-13:38, 13:40-13:42, 13:44-13:45, 13:49N, 13:53-13:56N, 13:58, 15:42, 35:60, 44:15, 44:18, 45:01, 45:03-45:08, 45:10-45:13, 46:18, 49:01:01-49:03, 49:06-49:17, 49:19N-49:22, 50:01:01-50:02, 50:04-50:08, 50:10-50:11, 50:13, 50:15, 54:07, 54:24, 55:18, 55:34, 55:47, 56:23		2						
Well No.	1	2	3	4	5	6	7	8

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Length of spec.	430	105	105	150	400	105	180	235
PCR product								
Well No.	1	2	3	4	5	6	7	8
*07:84, 08:09, 08:84, 13:35, 15:83, 27:14,								
27:81, 40:06:01:01-40:06:05, 40:44,								
40:53, 40:70, 40:75, 40:83, 40:93, 40:95-								
40:96, 40:103, 40:109-40:110, 40:127,								
40:131, 40:148, 40:161-40:162, 40:165,								
40:167, 40:177, 40:190, 41:01, 41:05-								
41:07, 41:09, 41:12, 41:14, 41:16-41:17,								
41:20-41:21, 42:04, 44:20, 44:47, 44:100,								
49:18, 50:14, 51:01:01-51:03, 51:05,			3					
51:07:01-51:12, 51:14, 51:16-51:24:04,								
51:26-51:34, 51:36, 51:38-51:41N, 51:43-51:44N, 51:48-51:55, 51:57-51:58, 51:60-								
51:61:02, 51:65-51:80, 51:82-51:91,								
51:93-51:96, 51:98N, 51:100-51:105,								
51:107-51:130, 51:132, 51:134, 51:136-								
51:137, 52:01:01:01-52:13, 52:15-52:24,								
52:26-52:30, 58:08:01-58:08:02, 78:01:01-								
78:07								
*13:03, 13:48, 45:14, 46:11, 51:15, 52:25,		2					7	
56:14, 56:17, 56:33							1	
*13:46, 15:137, 39:06:01-39:06:02, 39:33-								
39:34, 39:50, 39:57, 39:62, 39:64, 40:86,						6		
45:02, 50:09, 51:13:01-51:13:02, 51:92,								
52:14, 73:01-73:02							_	
*15:73, 40:71, 44:10, 51:62, 51:106					_		7	
*40:166, 81:03-81:04N					?			
*54:01:01-54:02, 54:05N, 54:08N, 54:10,								
54:13, 54:16-54:23, 54:25-54:27,								
55:01:01-55:01:03, 55:01:05-55:01:06,								
55:01:08-55:03, 55:05, 55:07, 55:09-		2			5			
55:12, 55:15-55:16, 55:19, 55:21-55:22,								
55:24-55:26, 55:29-55:31, 55:33, 55:35-								
55:38, 55:40-55:41, 55:43, 55:45-55:46, 55:48, 55:50, 55:52, 55:54-55:56, 56:19N								
Well No.	1	2	3	4	5	6	7	8
AACII IAO'	1		<u> </u>	+	J	U		O

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Length of spec.	430	105	105	150	400	105	180	235
PCR product								
Well No.	1	2	3	4	5	6	7	8
*54:03, 56:01:01-56:01:04, 56:07-56:08,		2			5		7	
56:13, 56:16, 56:20:01-56:20:02, 56:24- 56:30, 56:34-56:36		2) 		'	
*54:04, 54:11, 54:15, 55:17, 55:20, 55:27-55:28, 56:05:01-56:06, 56:15			3		5			
*54:12	1	2		4	5			
*55:13, 56:22					5	6		
*56:02, 56:04, 82:01-82:03					5		7	
*56:21	1		3		5			
HLA-B allele								
Well No.	1	2	3	4	5	6	7	8

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band.

²The nucleotide position, in the 1st, 2nd or 3rd exon, 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, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the

The nucleotide position, in the 2nd or 3rd exon, 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.

⁴HLA-B*59 alleles in bold lettering are listed as confirmed alleles on the on the IMGT/HLA web page www.ebi.ac.uk/imgt/hla, release 3.9.0, July 2012.

^{&#}x27;?', nucleotide sequence information for the primer matching region not known.

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CELL LINE VALIDATION SHEET												
		HLA-B	*59 SS	SP prim	er	se	t					
									ell			
					1	2	3	4	5	6	7	8
						O.	~	4	10	(0	_	~
				0	201206001	200624802	200624803	201081404	200964405	200850506	200964407	200964408
				Z .	90	24	24	8	64	50	64	64
				Prod. No.:	12	90	90	100	8	80	80	8
				7	20	8	8	2	8	8	8	8
	IHV	VC cell line		B*								
1	9001	SA	*07:02		-	-	-	-	-	-	-	-
2	9280	LK707	*52:01	*73:01	-	-	+	-	-	+	-	-
3	9011	E4181324	*52:01		-	-	+	-	-	-	-	-
4	9275	GU373	*15:10	*53:01	-	-	-	-	-	-	-	-
5	9009	KAS011	*37:01		-	-	-	-	-	-	-	-
6	9353	SM	*39:01	*51:01	-	-	+	-	-	-	-	-
7	9020		*18:01	01101	-	-	-	-	-	-	-	-
8	9025		*35:01		-	-	-	-	-	-	-	-
9	9026		*38:01		-	-	-	-	-	-	-	-
10		LKT3	*54:01		-			H		-	-	-
11		PITOUT	*44:03		-	+	-	-	+	H		
12	9051		*57:01			-	-		-	Ē	-	-
					-	-	-	-	-	-	-	-
13		JESTHOM	*27:05	*4= 00	-	-	-	-	-	-	-	-
14		OLGA	*15:01	*15:20	-	-	-	-	-	-	-	-
15	9075		*40:01		-	-	-	-	-	-	-	-
16		SWEIG007	*40:02		-	-	-	-	-	-	-	-
17		CTM3953540	*08:01	*55:01	-	+	-	-	+	-	-	-
18	9257	32367	*14:01	*56:01	-	+	-	-	+	-	+	-
19	9038	BM16	*18:01		-	-	-	-	-	-	-	-
20	9059	SLE005	*40:01		-	-	-	-	-	-	-	-
21	9064	AMALA	*15:01		-	-	-	-	-	-	-	-
22	9056	KOSE	*35:03		-	-	-	-	-	-	-	-
23	9124	IHL	*40:02	*56:02	-	-	-	-	+	-	+	-
24	9035	JBUSH	*38:01		-	-	-	-	-	-	-	-
25	9049	IBW9	*14:02		-	-	-	-	-	-	-	-
26	9285	WT49	*58:01		-	-	-	-	-	-	-	-
27	9191	CH1007	*07:05	*51:01	-	-	+	-	-	-	-	-
28		BEL5GB	*44:02	*44:03	-	-	-	-	-	-	-	-
29	9050		*44:03	11.00	-	-	-	-	-	-	-	-
30	9021		*42:01		-	_	_	-	_	-	_	_
31		DUCAF	*18:01		-	-	-	-	_	-	-	-
32	9297		*41:02		-	-	-	H	-	-	-	
							_		<u> </u>			-
33		MT14B	*40:01		-	-	-	-	-	-	-	-
34	9104		*38:01		-	-	-	-	-	-	-	-
35		SSTO	*44:02	*0F 01	-	-	-	-	-	-	-	-
36		KT17	*15:01	*35:01	-	-	-	-	-	-	-	-
37		HHKB	*07:02		-	-	-	-	-	-	-	-
38	9099		*15:01		-	-	-	-	-	-	-	-
39	9315		*08:01	*27:05	-	-	-	•	-	-	-	-
40	9134	WHONP199	*13:02	*46:01	-	+	-	-	-	-	-	-
41	9055	H0301	*14:02		-	-	-	-	-	-	-	-
42	9066	TAB089	*46:01		-	-	-	-	-	-	-	-
43	9076	T7526	*46:01		-	-	-	-	-	-	-	-
44	9057	TEM	*38:01		-	-	-	-	-	-	-	-
45		SHJO	*42:01	*50:01	-	+	-	-	-	-	-	-
46		SCHU	*07:02		-	-	-	-	-	-	-	-
47		TUBO	*51:01		-	-	+	-	-	-	-	-
48		TER-ND	*35:01	*44:03	-	-	-	-	-	-	-	-

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Lot No.: 18R Lot-specific information

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*59 SSP

Product number: 101.554-06 – including *Taq* pol.

101.554-06u - without Tag pol.

Lot number: 18R

Expiry date: 2015-March-01

Number of tests: 6 Number of wells per test: 8

Well specifications:

Well No.	Production No.
1	2012-060-01
2	2006-248-02
3	2006-248-03
4	2010-814-04
5	2009-644-06
6	2008-505-06
7	2009-644-07
8	2009-644-08

The specificity of each primer solution of the HLA-B*59 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 4 and 8 were available. The specificities of the primers in primer solution 4 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 8 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2012-September-21

Approved by:

Production Quality Control

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Lot No.: 18R Lot-specific information

Declaration of Conformity

Product name: Olerup SSP® HLA-B*59

Product number: 101.554-06/06u

Lot number: 18R

Intended use: HLA-B*59 high resolution histocompatibility testing

Manufacturer: Olerup SSP AB

Franzengatan 5

SE-112 51 Stockholm, Sweden

Phone: +46-8-717 88 27 **Fax:** +46-8-717 88 18

We, Olerup SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Stockholm, Sweden 2012-September-21

Ann-Cathrin Jareman Head of QA and Regulatory Affairs

Lot No.: 18R Lot-specific information

Visit <u>www.olerup-ssp.com</u> for "Instructions for Use" (IFU)



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