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Lot No.	A15-1


CTS Collaborative Transplant Study

WORKING INSTRUCTION

HLA-A* CTS-PCR-SSP TRAY KIT

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following product:

Product No.	Description
101	HLA-A* CTS-PCR-SSP TRAY KIT 

1. Main differences

- **Between Lot A15-1 (the current lot) and Lot A15-0**
 The kit was updated to cover new alleles included in the IMGT/HLA Database of January 2016. Deleted and renamed alleles were taken into consideration.
- **Attention**
 All CTS-PCR-SSP Kits (for both, HLA class I and HLA class II typing) can be used with the new "Mastermix SSP" as PCR buffer. There is no change in the Mastermix volume to be used for setting up the PCR reaction. Taq polymerase is also required as previously.

2. Introduction

- **Intended use:** This kit provides reagents for low/intermediate resolution HLA-A typing using the PCR-SSP method. All serologically detectable HLA-A alleles as well as their splits can be assigned. In addition, some of the DNA-specificities which so far could not be identified by serology can be detected.
- Allele coverage: IMGT/HLA Sequence Database Release 3.23.0, January 2016, except HLA-A*01:01:65-01:01:66/01:38/01:72/01:102/01:139/01:167, A*11:01:35/11:01:57/11:24-11:25:02/11:31/11:35/11:40/11:45/11:50Q/11:78N/11:111-11:112/11:176/11:183/11:191/11:211/11:223/11:226, A*24:129, A*25:01:09, A*26:60N/26:85, A*29:01:07/29:60, A*30:26/30:82/30:84, A*31:01:07/31:21-31:22/31:75/31:90, A*32:01:07/32:01:12/32:28/32:33:02-32:34/32:66/32:71, A*33:01:05/33:03:27/33:18:02/33:30/33:32:01-33:32:02/33:35/33:81/33:89, A*68:01:11/68:01:25/68:02:07/68:48/68:50/68:137, A*74:16:01, A*80:02
- Those alleles are considered to be rare and can be detected e. g. by sequencing with our CTS-SEQUENCE Kit (you may contact us for further information).
- This manual is only valid for **Lot No. A15-1**.
- This manual should be used together with the Main Manual (General Information) which is either the 'Working instruction for the CTS-PCR-SSP **TRAY and MINITRAY KITS**' (Manual No. 100A).

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Table 1: Sizes of the PCR products and allele specificities of each HLA-A* CTS-PCR-SSP primer mix (Lot No. A15-1) based on IMGT/HLA Sequence Database Release 3.23.0, January 2016	5
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Table 2: Amplification patterns for all detectable HLA-A* specificities (Lot-No A15-1) based on IMGT/HLA Sequence Database Release 3.23.0, January 2016.....	11
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4. Kit Composition

- Number of PCR primer mixes per test: 24 allele-specific mixes
- Number of tests per tray: 4
- Number of trays per kit: 10
- The primer mixes are aliquoted and lyophilized in thin-walled, red PCR trays.
- PCR buffer: 3.0 ml of Mastermix SSP (without Taq polymerase)

For storage condition, please refer to Section 1 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No.: 100A) supplied along with this product.

5. Materials, Reagents and Equipment not supplied

Please refer to Section 2 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No.: 100A) supplied along with this product.

6. Sample Requirements, PCR and Gel Electrophoresis

Please refer to Section 3 to 6 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No.: 100A) supplied along with this product.

7. Result Evaluation

- Check the approximate base pair size of the PCR product against the Primer Mix Specificity Table (Table 1) to confirm the correct product size.
- Use the Reaction Pattern Tables (Table 2) to make the allele assignments. Alternatively, you can use the SCORE Software (www.IHWG.org) for detailed result interpretation.

8. Interpretation Hints

- The quality and quantity of DNA as well as of the Taq polymerase are extremely crucial factors. If your bands are too weak, you might try to adjust these two factors until you obtain optimal results.
- Please also refer to Section 7 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINI-TRAY KITS' (Manual No.: 100A) supplied along with this product.

9. Special notes

- The following mixes will give **short** PCR products (<200 bp): Mixes **5, 17, 20, 23 and 24**. These PCR products might be difficult to distinguish from the 90 bp amplification control (internal positive control)!
In general, these specific products will give a much stronger signal than that of the controls. Furthermore, they will not have migrated as far into the gel as the control bands. If you are not sure whether the strong signal is due to a specific or a generic PCR product, you might let the gel run for additional 15 minutes at a lower voltage. By this way the specific band will be separated from the control band, and you will be able to clearly see a double signal at this position: a very strong specific band, and a weaker, shorter amplification control band.
- Since mix **5** corresponds to A*23 and mix **23** to A*02 (both alleles/allele groups are common), it is recommended to start the interpretation by checking these reactions very carefully first!
- Some mixes contain specific primers which may give rise to PCR fragments of **two or more** different sizes simultaneously (see Table 1).
- **Mix 4**: 225 bp and 235 bp (these two bands tend to merge into a single band). An additional weak band of 740 bp may occur, but is usually invisible.

10. Troubleshooting

Please refer to Section 8 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No.: 100A) supplied along with this product.

11. Precaution

Please refer to Material Safety Data Sheet for the CTS-PCR-SSP TRAY and MINITRAY KITS (Manual No.:100B) supplied along with this product.

12. Contact

If you have any particular questions concerning this kit, which are not answered in this or the Main Manual, please do not hesitate to contact me or my coworkers at:

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Hien Tran, M.D.

13. Appendix

Table 1: Sizes of the PCR products and allele specificities of each **HLA-A*** CTS-PCR-SSP primer mix (**Lot No. A15-1**) based on IMGT/HLA Sequence Database Release 3.23.0, January 2016

Position				Mix	Allele	Serology	Size
H1	H4	H7	H10	Mix 1	A*01:01:01:01-01:01:64/01:01:67-01:04N/01:06-01:33/01:35-01:37/01:39-01:71/01:73-01:101/01:103-01:138/01:140-01:166/01:168-01:199, A*11:27/11:38-11:39/11:94/11:209, A*23:31/23:45/23:55, A*24:15/24:41/24:51/24:92/24:235, A*26:29/26:49, A*32:13, A*66:10, B*07:64, B*15:12/15:19/15:270/15:298/15:304N	A1, Null, -, A26(10), B76(15)	225 bp
G1	G4	G7	G10	Mix 2	A*02:609w, A*03:107/03:164, A*11:17, A*26:72, A*32:24, A*36:01-36:05	-, A36	580 bp
F1	F4	F7	F10	Mix 3	A*02:01:01:01-02:01:15/02:01:18-02:01:19/02:01:21-02:01:25/02:01:27-02:01:51/02:01:53-02:01:63/02:01:65w/02:01:66-02:01:68/02:01:69w/02:01:70-02:01:73/02:01:75-02:01:80/02:01:83-02:01:94/02:01:95w/02:01:96-02:03:06/02:03:08-02:05:02/02:05:04-02:06:11/02:06:13-02:06:18/02:06:19w/02:06:20-02:11:06/02:11:07w/02:11:08-02:22:02/02:24:01-02:35:01/02:35:03-02:47/02:49/02:51/02:53N-02:54/02:56:01/02:57-02:63/02:66-02:71/02:73-02:79:02/02:81-02:87/02:89-02:97:02/02:99/02:101:01-02:102/02:104/02:105w/02:106-02:113:02N/02:115-02:116/02:118/02:120-02:121/02:123-02:128/02:130-02:134/02:136-02:151/02:153:01-02:168/02:170-02:177/02:179-02:184/02:186-02:187/02:189-02:192/02:193w/02:194/02:196-02:222N/02:224-02:228/02:230-02:245/02:247-02:272/02:273w/02:274/02:276-02:278/02:280-02:285/02:286w/02:287/02:289:01/02:290-02:297/02:299-02:300/02:302-02:303/02:305N-02:307/02:308w/02:310/02:311w/02:312-02:327/02:328w/02:329-02:338/02:340-02:347/02:349-02:376/02:378-02:382/02:384-02:405/02:407-02:411/02:413-02:453/02:455-02:487/02:488w/02:489-02:490N/02:492-02:503/02:505-02:509/02:511-02:526/02:528-02:570/02:572-02:581/02:583-02:590/02:592-02:600/02:602-02:610, A*24:02:76/24:310:02	A2, Low A2, -, A203, A210, Null	455 bp
E1	E4	E7	E10	Mix 4	A*01:13/01:176/01:194, A*03:01:01:01-03:193/03:195-03:242, A*11:130/11:199:01/11:222, A*30:55/30:89, A*32:04/32:17w/32:52, A*34:08, A*36:02, A*68:71, A*74:13	-, A3, Null	see below
					A*01:13/01:176/01:194, A*03:01:01:01-03:07/03:09-03:11N/03:13-03:31/03:33-03:35/03:37-03:40/03:42-03:71/03:73-03:87/03:90-03:106/03:109-03:151/03:153-03:171/03:173-03:175/03:177-03:193/03:195-03:197N/03:199-03:204/03:206-03:242, A*11:199:01/11:222, A*30:55, A*32:17w, A*34:08, A*68:71, A*74:13	-, A3, Null	225 bp
					A*03:01:01:01-03:04:03/03:07-03:09/03:11N/03:13-03:17:02/03:19-03:41/03:43-03:71/03:73-03:87/03:89:01-03:121/03:123:01-03:134/03:136-03:166/03:168N-03:186/03:188-03:193/03:195-03:204/03:206-03:224/03:226-03:242, A*32:04/32:52, A*36:02	A3, Null, -	740 bp (weak or absent)
					A*03:01:01:01-03:04:03/03:07-03:09/03:11N-03:17:02/03:19-03:41/03:43-03:74/03:76-03:94/03:96-03:121/03:123:01-03:134/03:136-03:166/03:168N-03:176/03:178N-03:186/03:188-03:193/03:195-03:203/03:205-03:214/03:216-03:224/03:226-03:242, A*11:130, A*30:89, A*32:04, A*36:02	A3, Null, -	235 bp
D1	D4	D7	D10	Mix 5	A*23:01:01-23:23/23:25-23:33/23:35-23:56/23:58-23:65/23:67-23:69, A*24:24/24:71/24:315, A*29:07/29:49, A*31:29, B*18:27	A23(9), -, Null	195 bp

Position				Mix	Allele	Serology	Size
C1	C4	C7	C10	Mix 6	A*02:46/02:48/02:70/02:129/02:571, A*03:30/03:152, A*23:01:01-23:02/23:04-23:08N/23:10-23:22/23:24-23:27/23:29-23:36/23:38N-23:50/23:52-23:60/23:62-23:69, A*24:02:01:01-24:02:16/24:02:18-24:02:48/24:02:50-24:02:63/24:02:64w/24:02:65/24:02:66w/24:02:67-24:02:88/24:02:90-24:07:02/24:09N-24:11N/24:13:01-24:15/24:17-24:20/24:22-24:23/24:25-24:28/24:30/24:32-24:41/24:43-24:64/24:66/24:68-24:76/24:78-24:88/24:90:01N-24:128/24:130-24:155N/24:157/24:159-24:190/24:192-24:199/24:201-24:210/24:212-24:236/24:238-24:272/24:274-24:289/24:291-24:308/24:311-24:325/24:327-24:334, A*31:08, A*32:79, A*33:21/33:53	A2, -, A23(9), Null, A24(9), Low A24(9), A2403, A9, A24(9)/A3	see below
					A*03:30/03:152, A*23:31/23:45/23:53, A*24:17/24:41/24:62/24:106/24:208/24:296/24:330, A*31:08, A*32:79, A*33:21/33:53	-, A24(9)	435 bp
					A*02:46/02:48/02:70/02:129/02:571, A*23:01:01-23:02/23:04-23:08N/23:10-23:22/23:24-23:27/23:29-23:36/23:38N-23:50/23:52-23:60/23:62-23:69, A*24:02:01:01-24:02:16/24:02:18-24:02:48/24:02:50-24:02:63/24:02:64w/24:02:65/24:02:66w/24:02:67-24:02:88/24:02:90-24:07:02/24:09N-24:11N/24:13:01-24:15/24:17-24:20/24:22-24:23/24:25-24:28/24:30/24:32-24:41/24:43-24:64/24:66/24:68-24:76/24:78-24:88/24:90:01N-24:128/24:130-24:155N/24:157/24:159-24:190/24:192-24:199/24:201-24:207/24:209-24:210/24:212-24:236/24:238-24:272/24:274-24:289/24:291-24:308/24:311-24:325/24:327-24:334	A2, -, A23(9), Null, A24(9), Low A24(9), A2403, A9, A24(9)/A3	470 bp
B1	B4	B7	B10	Mix 7	A*11:178/11:190, A*24:02:01:01-24:02:73/24:02:75-24:03:03/24:05/24:07:01-24:11N/24:14-24:15/24:17/24:20-24:21:03/24:23/24:25-24:27/24:29-24:43/24:45N-24:64/24:66-24:86N/24:88/24:90:01N-24:93/24:95-24:106/24:108/24:110-24:128/24:130-24:137/24:139-24:166/24:168-24:187/24:189-24:206/24:208-24:210/24:212-24:226:02/24:229-24:277/24:278Nw/24:279-24:284/24:286-24:288/24:291-24:298/24:300-24:314/24:316-24:334, A*25:04, A*32:02/32:22, B*51:55	-, A24(9), Low A24(9), A2403, A9, Null, A32(19)	510 bp
A1	A4	A7	A10	Mix 8	A*02:52/02:135/02:309/02:454, A*25:01:01/25:01:02w/25:01:03-25:01:08/25:02-25:10/25:11w/25:12N-25:23/25:25-25:32, A*30:01:01-30:04:01/30:06-30:20/30:22-30:25/30:27N-30:81/30:83/30:85-30:89/30:91-30:95/30:97, B*07:225w/07:260w, C* 04:01:49	-, A25(10), Null, A30(19)	see below
					A*02:52, A*30:01:01-30:02:09/30:02:11-30:04:01/30:06-30:20/30:22-30:25/30:27N-30:81/30:83/30:85-30:89/30:91-30:95/30:97	-, A30(19), Null	550 bp
					A*02:135/02:309/02:454, A*30:02:10, C* 04:01:49	-	560 bp
					B*07:225w/07:260w	-	395 bp
					A*25:01:01/25:01:02w/25:01:03-25:01:08/25:02-25:10/25:11w/25:12N-25:23/25:25-25:32	A25(10), -, Null	405 bp

Position				Mix	Allele	Serology	Size
H2	H5	H8	H11	Mix 9	A*02:135/02:309/02:454, A*25:01:01-25:01:07/25:01:08w/25:02-25:13/25:15-25:33, A*26:01:01:01-26:01:26/26:01:28-26:01:37/26:01:38w/26:01:39-26:18/26:20-26:30/26:32-26:43:02/26:45-26:59/26:61-26:71N/26:73-26:75/26:76w/26:77-26:83/26:86-26:120, A*34:01:01-34:11/34:13-34:14, A*66:01:01-66:22/66:24, B*38:02:04	-, A25(10), Null, A26(10), A10, A34(10), A66(10)	see below
					A*02:135/02:309/02:454, A*26:07:01-26:07:02/26:92	-, A26(10)	450 bp
					A*25:01:01-25:01:07/25:01:08w/25:02-25:13/25:15-25:33, A*26:01:01:01-26:01:26/26:01:28-26:01:37/26:01:38w/26:01:39-26:06/26:08-26:18/26:20-26:30/26:32-26:43:02/26:45-26:59/26:61-26:71N/26:73-26:75/26:76w/26:77-26:83/26:86-26:91/26:93-26:120, A*34:01:01-34:11/34:13-34:14, A*66:01:01-66:22/66:24, B*38:02:04	A25(10), -, Null, A26(10), A10, A34(10), A66(10)	445 bp
G2	G5	G8	G11	Mix 10	A*01:01:56, A*02:135/02:309/02:454, A*25:11, A*26:01:01:01-26:01:01:02/26:01:03-26:01:12/26:01:14-26:32/26:34-26:43:02/26:45-26:59/26:61-26:84/26:86-26:109/26:111-26:120, A*30:02:10, A*43:01	-, A26(10), A10, Null, A43	see below
					A*02:135/02:309/02:454, A*25:11, A*26:03:01/26:06/26:21/26:30/26:36/26:78/26:92/26:111	-, A26(10)	415 bp
					A*01:01:56, A*26:01:01:01-26:01:01:02/26:01:03-26:01:12/26:01:14-26:02:02/26:04-26:05/26:07:01-26:20/26:22-26:29/26:31-26:32/26:34-26:43:02/26:45-26:59/26:61-26:77/26:79-26:84/26:86-26:91/26:93-26:109/26:112-26:120, A*30:02:10, A*43:01	-, A26(10), A10, Null, A43	405 bp
F2	F5	F8	F11	Mix 11	A*02:17:01-02:17:03/02:108/02:110/02:268w/02:300/02:303, A*11:139, A*23:04, A*24:03:01-24:03:03/24:10:01-24:10:02/24:18/24:22/24:33/24:94/24:125/24:138/24:167/24:204/24:207/24:210/24:289/24:299-24:300/24:315, A*26:48/26:69, A*29:07/29:49, A*31:29, A*34:01:01-34:01:02/34:05/34:11-34:12/34:14, B*18:27w	A2, -, A23(9), A2403, A24(9)/A3, A9, A34(10)	see below
					A*02:300/02:303, A*24:03:03/24:10:02/24:125/24:138/24:167/24:204/24:207/24:210/24:289/24:299-24:300/24:315, A*26:48/26:69, A*34:01:01-34:01:02/34:05/34:11-34:12/34:14	-, A34(10)	580 bp
					A*02:17:01-02:17:03/02:108/02:110/02:268w/02:300/02:303, A*11:139, A*23:04, A*24:03:01-24:03:03/24:10:01-24:10:02/24:18/24:22/24:33/24:94/24:125/24:138/24:167/24:204/24:207/24:210/24:289/24:299-24:300/24:315, A*29:07/29:49, A*31:29, B*18:27w	A2, -, A23(9), A2403, A24(9)/A3, A9	245 bp
E2	E5	E8	E11	Mix 12	A*03:103, A*11:116/11:140, A*25:02, A*26:13/26:19/26:33, A*29:32, A*31:89, A*32:17, A*34:01:01-34:01:02/34:05-34:06/34:11-34:12/34:14, A*66:01:01-66:02/66:04/66:06-66:14/66:16-66:24, A*74:13	-, A25(10), A34(10), A66(10)	415 bp
D2	D5	D8	D11	Mix 13	A*01:51, A*03:24, A*11:10, A*33:49, A*34:02:01-34:04/34:07-34:10N/34:13, A*68:103:01-68:103:02	-, A3, A11, A34(10), Null	385 bp

Position				Mix	Allele	Serology	Size
C2	C5	C8	C11	Mix 14	A*02:12-02:13/02:19/02:27/02:37-02:38/02:44/02:49/02:54/02:142/02:226N/02:271/02:280/02:417/02:543, A*03:02:01w-03:02:04w/03:10/03:31w-03:32w/03:65w/03:69Nw/03:73w/03:76w/03:82w/03:90w/03:106w/03:113w/ 03:160w/03:167/03:198w/03:218w/03:223w/03:225/03:236w-03:237w/03:242w, A*11:01:01-11:01:34/11:01:36- 11:01:56/11:01:58-11:16/11:18-11:22/11:23w/11:26-11:27/11:29-11:30/11:32:01-11:34/11:36-11:39/11:41-11:44/ 11:46-11:49/11:51-11:52Q/11:54-11:77/11:79-11:93/11:95-11:97/11:98w/11:99N-11:110/11:113-11:129/11:130w/ 11:131-11:157/11:159-11:175/11:177/11:179-11:182Q/11:184-11:189/11:192-11:210N/11:212-11:222/11:224-11:225/ 11:227-11:228/11:229w/11:230-11:235Q, A*24:19/24:28/24:44/24:89/24:290, A*31:24w/31:97w, A*33:19w/33:59w/ 33:102w, A*66:19, A*68:09/68:26/68:28/68:129/68:134, A*69:03	A2, -, Null, A3, A11, A9, A24(9), A28	520 bp
B2	B5	B8	B11	Mix 15	A*03:01:39, A*29:01:01-29:01:06/29:01:08-29:59/29:61-29:83, A*32:30/32:32, A*68:01:28, A*74:10	-, A29(19), Null	see below
					A*29:01:01-29:01:06/29:01:08-29:12/29:14-29:18/29:20-29:38/29:40-29:47/29:49-29:59/29:61-29:83, A*74:10	A29(19), Null, -	160 bp
					A*03:01:39, A*29:01:01-29:01:06/29:01:08/29:02:01-29:31/29:33-29:59/29:61-29:83, A*32:30/32:32, A*68:01:28	-, A29(19), Null	515 bp
A2	A5	A8	A10	Mix 16	A*30:01:01-30:01:07/30:01:08w/30:01:09-30:02:09/30:02:11-30:04:02/30:06-30:11:02/30:13-30:17/30:19-30:20/ 30:22-30:25/30:27N-30:40/30:42-30:54/30:56-30:81/30:83/30:85-30:97	A30(19), -, Null	570 bp
H3	H6	H9	H12	Mix 17	A*29:14, A*31:01:02-31:01:06/31:01:08-31:07/31:09-31:20/31:23-31:74/31:76-31:88/31:91-31:103/31:104w/ 31:105	-, A31(19), Null	185 bp
G3	G6	G9	G12	Mix 18	A*03:152/03:219, A*23:03:01, A*24:18/24:21:03/24:204/24:208/24:213, A*29:13, A*31:07-31:08/31:10, A*32:01:01- 32:01:06/32:01:08-32:01:11/32:01:13-32:27N/32:29-32:33:01/32:35-32:65/32:67-32:70/32:72-32:74/32:75w/32:76- 32:84	-, A24(9)/A3, A31(19), A32(19), Null	see below
					A*23:03:01, A*24:21:03/24:208, A*29:13, A*31:07-31:08/31:10, A*32:01:01-32:01:06/32:01:08-32:01:11/32:01:13- 32:03/32:05-32:27N/32:29-32:33:01/32:35-32:65/32:67-32:70/32:72-32:74/32:75w/32:76-32:84	-, A31(19), A32(19), Null	410 bp
					A*03:152/03:219, A*24:18/24:204/24:213, A*32:04/32:52	-, A24(9)/A3	520 bp
F3	F6	F9	F12	Mix 19	A*02:24:02/02:65/02:152/02:507, A*29:48, A*32:15, A*33:01:01-33:01:04/33:01:06-33:01:08/33:03:01-33:03:25/ 33:03:26w/33:03:28-33:18:01/33:20/33:22-33:29/33:31/33:33-33:34/33:36-33:37/33:39-33:52/33:54-33:65/33:66w/ 33:67-33:80N/33:82-33:88/33:90-33:106, A*74:04/74:21, B*07:02:40, B*08:01:07, B*15:02:07, B*55:02:10	-, A2/19, A33(19), A19, Null, B8	see below
					A*02:24:02/02:65/02:152/02:507, A*29:48, A*33:08-33:09, A*74:04/74:21	-, A2/19, A33(19), A19	475 bp
					A*32:15, A*33:01:01-33:01:04/33:01:06-33:01:08/33:03:01-33:03:25/33:03:26w/33:03:28-33:07/33:10-33:18:01/ 33:20/33:22-33:29/33:31/33:33-33:34/33:36-33:37/33:39-33:52/33:54-33:65/33:66w/33:67-33:80N/33:82-33:88/ 33:90-33:106, B*07:02:40, B*08:01:07, B*15:02:07, B*55:02:10	-, A33(19), Null, B8	470 bp
E3	E6	E9	E12	Mix 20	A*03:01:39, A*29:19/29:39/29:48, A*68:01:28, A*74:01:01-74:09/74:11-74:15/74:16:02-74:27	-, A74(19), Null	160 bp

Position				Mix	Allele	Serology	Size
D3	D6	D9	D12	Mix 21	A*02:01:09/02:05:05/02:06:07/02:11:02/02:35:03/02:50/02:76:02/02:122/02:243:02/02:570/02:591, A*03:171, A*11:57/11:158, A*24:94/24:138/24:188/24:228/24:293, A*25:05, A*26:54, A*29:51/29:69/29:73, A*68:01:01:01-68:01:10/68:01:12-68:01:24/68:01:26-68:02:06/68:02:08-68:47/68:49Nw/68:51-68:58/68:59Nw/68:60-68:128/68:130-68:136/68:138-68:142N, A*69:01-69:03	A2, -, A68(28), A28, Null, A69(28)	645 bp
C3	C6	C9	C12	Mix 22	A*02:34-02:35:03/02:56:01-02:56:02/02:62/02:78/02:103, A*03:241, A*69:01-69:03	A2, -, A69(28)	390 bp
B3	B6	B9	B12	Mix 23	A*02:01:01:01-02:01:08/02:01:10-02:01:11/02:01:12w/02:01:13-02:01:15/02:01:17-02:01:19/02:01:21-02:01:32/02:01:33w/02:01:34-02:01:39/02:01:41-02:01:81/02:01:83-02:05:04/02:05:06-02:06:06/02:06:08-02:11:01/02:11:03-02:22:02/02:24:01-02:35:01/02:35:02w/02:36-02:76:01/02:77/02:79:01-02:97:02/02:99/02:101:01-02:121/02:123-02:140/02:142-02:243:01/02:244-02:311/02:313-02:469/02:471-02:569/02:571-02:590/02:592-02:610, A*03:89:01, A*24:02:06, A*30:07w/30:13w/30:16w/30:22w/30:44w/30:46w, B*51:01:34w, C*07:02:27w	A2, Low A2, -, A203, A210, Null, A2/19, A24(9)	see below
					A*02:143/02:556	-	175 bp
					A*02:50/02:143/02:556	A2, -	205 bp
					A*02:50/02:143/02:556	A2, -	225 bp
					A*02:01:01:01-02:01:08/02:01:10-02:01:11/02:01:12w/02:01:13-02:01:15/02:01:18-02:01:19/02:01:21-02:01:32/02:01:33w/02:01:34-02:01:39/02:01:41-02:01:51/02:01:53-02:01:73/02:01:75-02:01:81/02:01:83-02:05:02/02:05:04/02:05:06-02:06:06/02:06:08-02:06:11/02:06:13-02:11:01/02:11:03-02:22:02/02:24:01-02:35:01/02:36-02:47/02:49/02:51-02:54/02:56:01/02:57-02:60:02/02:62-02:76:01/02:77/02:79:01-02:97:02/02:99/02:101:01-02:102/02:104-02:121/02:123-02:128/02:130-02:140/02:142-02:143/02:145-02:168/02:170-02:175/02:177-02:184/02:186-02:194/02:196-02:204/02:206-02:243:01/02:244-02:311/02:313-02:314N/02:316-02:377/02:379-02:469/02:471-02:481/02:483-02:503/02:505-02:569/02:572-02:590/02:592-02:600/02:602-02:610, A*03:89:01, A*30:22w, B*51:01:34w, C*07:02:27w	A2, Low A2, -, A203, A210, Null, A2/19	135 bp
A*02:01:01:01-02:01:08/02:01:10-02:01:11/02:01:12w/02:01:13-02:01:15/02:01:17-02:01:19/02:01:21-02:01:30/02:01:32/02:01:33w/02:01:34-02:01:39/02:01:41-02:01:81/02:01:83-02:05:04/02:05:06-02:06:06/02:06:08-02:07:08/02:09-02:11:01/02:11:03-02:19/02:21-02:22:02/02:24:01-02:35:01/02:35:02w/02:36-02:49/02:51-02:54/02:57-02:61/02:63-02:76:01/02:77/02:79:01-02:97:02/02:99/02:101:01-02:102/02:104-02:121/02:123-02:127/02:129-02:140/02:142-02:168/02:170-02:194/02:196-02:243:01/02:244-02:271/02:273-02:311/02:313-02:414/02:416-02:469/02:471-02:569/02:571-02:590/02:592-02:608N/02:610, A*03:89:01, A*24:02:06, A*30:07w/30:22w	A2, Low A2, -, A203, A210, Null, A2/19, A24(9)	165 bp					
A*02:01:01:01-02:01:02/02:01:04-02:01:08/02:01:10-02:01:11/02:01:12w/02:01:13-02:01:15/02:01:17-02:01:19/02:01:21-02:01:32/02:01:33w/02:01:34-02:01:39/02:01:41-02:01:81/02:01:83-02:05:04/02:05:06-02:06:06/02:06:08-02:10/02:12-02:22:02/02:24:01-02:34/02:36-02:47/02:49/02:51-02:68/02:70-02:76:01/02:77/02:79:01-02:80/02:82N-02:86/02:88N-02:89/02:91-02:97:02/02:99/02:101:01-02:111/02:113:01N-02:121/02:123/02:125N-02:127/02:130-02:135/02:137-02:140/02:142-02:145/02:147-02:243:01/02:244-02:246/02:248-02:296/02:299-02:307/02:309-02:311/02:313-02:330/02:332-02:456/02:458-02:469/02:471-02:489/02:491-02:493/02:495-02:502/02:504-02:513/02:515-02:569/02:572-02:578/02:581-02:583/02:585-02:588/02:590/02:592-02:610, A*03:89:01, A*30:13w/30:16w/30:44w/30:46w	A2, Low A2, -, A203, A210, Null, A2/19	190 bp					

Position				Mix	Allele	Serology	Size
A3	A6	A9	A12	Mix 24	A*43:01, A*80:01:01:01-80:01:01:02/80:03	A43, A80, -	see below
					A*80:01:01:01-80:01:01:02/80:03	A80, -	170 bp
					A*43:01	A43	445 bp

Amplification control (internal positive control): 90 base pairs (bp)

w = weak

? = nucleotide sequence information not available for the primer matching sequence

Bold: mixes which result in PCR fragments of different sizes (the specificities are first indicated all in one row, then split into several groups in the subsequent rows depending on the fragment size)

Table 2: Amplification patterns for all detectable HLA-A* specificities (Lot-No A15-1) based on IMGT/HLA Sequence Database Release 3.23.0, January 2016

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A*01:01:01:01:01:01:55/01:01:57-01:01:64/01:01:67-01:04N/01:06-01:12/01:14-01:33/01:35-01:37/01:39-01:50/01:52:01N-01:71/01:73-01:101/01:103-01:138/01:140-01:166/01:168-01:175/01:177-01:193/01:195-01:199, A*11:94, B*07:64, B*15:12/15:19/15:270/15:298/15:304N	A1, Null, -, B76(15)	1																								
A*01:01:56	-	1									10															
A*01:13/01:176/01:194	-	1			4																					
A*01:51	-	1												13												
A*02:01:01:01:02:01:08/02:01:10-02:01:11/02:01:13-02:01:15/02:01:18-02:01:19/02:01:21-02:01:25/02:01:27-02:01:32/02:01:34-02:01:39/02:01:41-02:01:51/02:01:53-02:01:63/02:01:66-02:01:68/02:01:70-02:01:73/02:01:75-02:01:80/02:01:83-02:01:94/02:01:96-02:03:06/02:03:08-02:05:02/02:05:04/02:05:06-02:06:06/02:06:08-02:06:11/02:06:13-02:06:18/02:06:20-02:11:01/02:11:03-02:11:06/02:11:08-02:11:09/02:14-02:16/02:18/02:20:01-02:22:02/02:24:01/02:25-02:26/02:28-02:33/02:36/02:39-02:43N/02:45/02:47/02:51/02:53N/02:57-02:61/02:63/02:66-02:69/02:71/02:73-02:76:01/02:77/02:79:01-02:79:02/02:81-02:87/02:89-02:97:02/02:99/02:101:01-02:102/02:104/02:106-02:107/02:109/02:111-02:113:02N/02:115-02:116/02:118/02:120-02:121/02:123-02:128/02:130-02:134/02:136-02:140/02:143-02:151/02:153:01-02:168/02:170-02:177/02:179-02:184/02:186-02:187/02:189-02:192/02:194/02:196-02:222N/02:224-02:225N/02:227N-02:228/02:230-02:243:01/02:244-02:245/02:247-02:267/02:269-02:270/02:272/02:274/02:276-02:278/02:281-02:285/02:287/02:289:01/02:290-02:297/02:299/02:302/02:305N-02:307/02:310/02:313-02:327/02:329-02:338/02:340-02:347/02:349-02:376/02:378-02:382/02:384-02:405/02:407-02:411/02:413-02:416/02:418-02:453/02:455-02:469/02:471-02:487/02:489-02:490N/02:492-02:503/02:505-02:506N/02:508-02:509/02:511-02:526/02:528-02:542/02:544-02:569/02:572-02:581/02:583-02:590/02:592-02:600/02:602-02:608N/02:610	A2, Low A2, -, A203, A210, Null			3																						23
A*02:01:09/02:05:05/02:06:07/02:11:02/02:76:02/02:243:02/02:570	A2, -			3																		21				
A*02:01:12/02:01:33	A2			3																					w	

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A*02:01:17/02:01:26/02:01:52/02:01:64/02:01:74/02:01:81/02:03:07/ 02:05:03/02:06:12/02:55/02:64:01-02:64:02/02:72/02:80/02:88N/02:114/ 02:117/02:119/02:169/02:178/02:185/02:188/02:195/02:223N/02:229/ 02:246/02:275/02:279/02:288/02:289:02/02:298/02:301N/02:304/02:339/ 02:348/02:377/02:383/02:406/02:412/02:491/02:504/02:510/02:527/ 02:582/02:601	A2, -, Null																							23	
A*02:01:40/02:141/02:312/02:470	A2, -			3																					
A*02:01:65/02:01:69/02:01:95/02:06:19/02:11:07/02:105/02:193/02:273/ 02:286/02:308/02:311/02:328/02:488	-, A2			w																				23	
A*02:12-02:13/02:19/02:27/02:37-02:38/02:44/02:49/02:54/02:142/ 02:226N/02:271/02:280/02:417/02:543	A2, -, Null			3											14									23	
A*02:17:01-02:17:03/02:108/02:110/02:300/02:303	A2, -			3							11													23	
A*02:24:02/02:507	-			3															19					23	
A*02:34-02:35:01/02:56:01/02:62	A2, -			3																			22	23	
A*02:35:02	A2																					22	w		
A*02:35:03	A2			3																	21	22			
A*02:46/02:70	A2			3			6																	23	
A*02:48/02:129/02:571	-						6																	23	
A*02:50	A2																					21	23		
A*02:52	-								8															23	
A*02:56:02/02:103	-																						22	23	
A*02:65/02:152	A2/19, -																			19				23	
A*02:78	A2			3																			22		
A*02:122/02:591, A*11:158, A*68:01:01:01-68:01:10/68:01:12-68:01:24/ 68:01:26-68:01:27/68:01:29-68:02:06/68:02:08-68:08:02/68:10-68:25/ 68:27:01-68:27:02/68:29-68:47/68:51-68:58/68:60-68:70/68:72-68:102/ 68:104:01-68:128/68:130-68:133/68:135-68:136/68:138-68:142N	-, A68(28), A28, Null																					21			
A*02:135/02:309/02:454	-								8	9	10													23	
A*02:268	-			3								w												23	
A*02:609	-		w	3																				23	

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A*03:01:01:01-03:01:38/03:01:40-03:01:56/03:03N-03:09/03:11N-03:23:02/ 03:25-03:29/03:33-03:64/03:66-03:68N/03:70-03:72/03:74-03:75/03:77- 03:81/03:83-03:88/03:89:02/03:91N-03:102/03:104-03:105/03:108-03:112/ 03:114-03:151/03:153-03:159/03:161N-03:163/03:165-03:166/03:168N- 03:170/03:172-03:193/03:195-03:197N/03:199-03:217/03:220-03:222/ 03:224/03:226-03:235/03:238-03:240	A3, Null, -				4																					
A*03:01:39	-				4											15					20					
A*03:02:01-03:02:04/03:31-03:32/03:65/03:69N/03:73/03:76/03:82/03:90/ 03:106/03:113/03:160/03:198/03:218/03:223/03:236-03:237/03:242, A*11:130	A3, -, Null				4										w											
A*03:10/03:167/03:225, A*11:199:01/11:222	-				4										14											
A*03:24	A3				4								13													
A*03:30	-				4		6																			
A*03:89:01	-				4																			23		
A*03:103	-				4							12														
A*03:107/03:164, A*36:02	-		2		4																					
A*03:152	-				4		6												18							
A*03:171, A*68:71	-				4																	21				
A*03:219, A*32:04/32:52	-				4														18							
A*03:241	-				4																		22			
A*11:01:01:01-11:01:34/11:01:36-11:01:56/11:01:58-11:09/11:11-11:16/ 11:18-11:22/11:26/11:29-11:30/11:32:01-11:34/11:36-11:37/11:41-11:44/ 11:46-11:49/11:51-11:52Q/11:54-11:56/11:58-11:77/11:79-11:93/11:95- 11:97/11:99N-11:110/11:113-11:115N/11:117-11:129/11:131-11:138/ 11:141-11:157/11:159-11:175/11:177/11:179-11:182Q/11:184-11:189/ 11:192-11:198/11:199:02-11:208N/11:210N/11:212-11:221/11:224-11:225/ 11:227-11:228/11:230-11:235Q, A*24:89/24:290, A*68:129	A11, -, Null														14											
A*11:10	A11												13	14												
A*11:17, A*36:01/36:03-36:05	-, A36		2																							
A*11:23/11:98/11:229, A*33:19	-														w											
A*11:27/11:38-11:39/11:209	-		1												14											
A*11:57, A*68:09/68:26/68:28/68:134	-, A28														14							21				
A*11:116/11:140	-											12	14													

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A*11:139	-											11			14										
A*11:178/11:190, A*24:02:17/24:02:49/24:02:89/24:08/24:21:01-24:21:02/24:29/24:31/24:42/24:67/24:77/24:156/24:158N/24:191/24:200/24:237/24:273/24:309-24:310:01/24:326, B*51:55	-, A24(9), Null							7																	
A*23:01:01-23:02/23:05-23:08N/23:10-23:22/23:25-23:27/23:29-23:30/23:32-23:33/23:35-23:36/23:38N-23:44/23:46-23:50/23:52-23:54/23:56/23:58-23:60/23:62-23:65/23:67-23:69	A23(9), -, Null					5	6																		
A*23:03:01	-					5													18						
A*23:03:02/23:09/23:23/23:28/23:37:01-23:37:02/23:51/23:61, A*24:24	-					5																			
A*23:04, A*24:315	A23(9), -					5	6					11													
A*23:24/23:34/23:57/23:66, A*24:02:74/24:04/24:06/24:13:01-24:13:02/24:87/24:107/24:109/24:227/24:285, A*33:21/33:53	-, A24(9)						6																		
A*23:31/23:45/23:55	-	1				5	6																		
A*24:02:01:01-24:02:05/24:02:07-24:02:16/24:02:18-24:02:48/24:02:50-24:02:63/24:02:65/24:02:67-24:02:73/24:02:75/24:02:77-24:02:88/24:02:90-24:02:96/24:05/24:07:01-24:07:02/24:09N/24:11N/24:14/24:17/24:20/24:23/24:25-24:27/24:30/24:32/24:34-24:40N/24:43/24:45N-24:50/24:52-24:64/24:66/24:68-24:70/24:72-24:76/24:78-24:86N/24:88/24:90:01N-24:91/24:93/24:95-24:106/24:108/24:110-24:124/24:126-24:128/24:130-24:137/24:139-24:155N/24:157/24:159-24:166/24:168-24:187/24:189-24:190/24:192-24:199/24:201-24:203/24:205-24:206/24:209/24:212/24:214-24:226:02/24:229-24:234/24:236/24:238-24:272/24:274-24:277/24:279-24:284/24:286-24:288/24:291-24:292/24:294Q-24:298/24:301-24:308/24:311-24:314/24:316-24:325/24:327-24:334	A24(9), Low A24(9), -, A9, Null, A2403						6	7																	
A*24:02:06	A24(9)						6	7																23	
A*24:02:64/24:02:66	-						w	7																	
A*24:02:76	-			3			6	7																	
A*24:03:01-24:03:03/24:10:01-24:10:02/24:33/24:125/24:210/24:300	A2403, -						6	7				11													
A*24:15/24:41/24:51/24:92/24:235	-	1					6	7																	
A*24:18	A24(9)/A3						6					11							18						
A*24:19/24:28/24:44	A9, A24(9), -						6								14										
A*24:21:03, A*32:02/32:22	-, A32(19)							7											18						
A*24:22/24:167/24:207/24:289/24:299	A9, -						6					11													
A*24:71	-					5	6	7																	

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A*24:94/24:138	-						6					11										21			
A*24:188/24:228	-						6															21			
A*24:204	-						6	7				11							18						
A*24:208/24:213	-						6	7											18						
A*24:278N	Null						6	w																	
A*24:293	-						6	7														21			
A*24:310:02	-			3				7																	
A*25:01:01/25:01:03-25:01:07/25:03/25:06-25:10/25:12N-25:13/25:15-25:23/25:25-25:32	A25(10), -, Null								8	9															
A*25:01:02	A25(10)								w	9															
A*25:01:08	-								8	w															
A*25:02	A25(10)								8	9		12													
A*25:04	-							7	8	9															
A*25:05	-								8	9												21			
A*25:11	-								w	9	10														
A*25:14, A*30:12/30:18/30:41, C* 04:01:49	-, A30(19)								8																
A*25:24/25:33, A*26:01:02/26:01:13/26:110, A*66:03/66:05/66:15, B*38:02:04	-, A26(10), A10									9															
A*26:01:01:01-26:01:01:02/26:01:03-26:01:12/26:01:14-26:01:26/26:01:28-26:01:37/26:01:39-26:12/26:14-26:18/26:20-26:28/26:30/26:32/26:34-26:43:02/26:45-26:47/26:50-26:53/26:55-26:59/26:61-26:68/26:70-26:71N/26:73-26:75/26:77-26:83/26:86-26:109/26:111-26:120	A26(10), -, A10, Null									9	10														
A*26:01:27/26:31/26:84	-										10														
A*26:01:38/26:76	-									w	10														
A*26:13	-									9	10	12													
A*26:19	-										10	12													
A*26:29/26:49	A26(10), -	1								9	10														
A*26:33, A*34:06, A*66:01:01-66:02/66:04/66:06-66:09/66:11-66:14/66:16-66:18/66:20-66:22/66:24	-, A66(10)									9		12													
A*26:48/26:69	-									9	10	11													
A*26:54	-									9	10											21			
A*26:72	-		2								10														

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A*29:01:01:01-29:01:06/29:01:08-29:06/29:08N-29:12/29:15-29:18/29:20-29:31/29:33-29:38/29:40-29:47/29:50/29:52-29:59/29:61-29:68/29:70-29:72/29:74-29:83, A*74:10	A29(19), Null, -															15									
A*29:07/29:49	-					5						11				15									
A*29:13, A*32:30/32:32	-															15		18							
A*29:14	-															15	17								
A*29:19/29:39	-															15				20					
A*29:32	-												12			15									
A*29:48	-															15			19	20					
A*29:51/29:69/29:73	-															15					21				
A*30:01:01:30:01:07/30:01:09-30:02:09/30:02:11-30:04:01/30:06/30:08-30:11:02/30:14L-30:15/30:17/30:19-30:20/30:23-30:25/30:27N-30:40/30:42-30:43/30:45/30:47-30:54/30:56-30:81/30:83/30:85-30:88/30:91-30:95/30:97	A30(19), -, Null								8								16								
A*30:01:08	-								8								w								
A*30:02:10	-								8		10														
A*30:04:02/30:90/30:96	-																16								
A*30:07/30:13/30:16/30:22/30:44/30:46	-								8								16						w		
A*30:55	-				4				8																
A*30:89	-				4				8								16								
A*31:01:02:01-31:01:06/31:01:08-31:06/31:09/31:11-31:20/31:23/31:25-31:28/31:30-31:74/31:76-31:88/31:91-31:96/31:98-31:103/31:105	A31(19), -, Null																	17							
A*31:07/31:10	-, A31(19)																	17	18						
A*31:08, A*32:79	-						6												18						
A*31:24/31:97	-														w			17							
A*31:29	-					5						11						17							
A*31:89, A*66:23	-												12												
A*31:104	-																	w							
A*32:01:01-32:01:06/32:01:08-32:01:11/32:01:13-32:01:23/32:03/32:05-32:12/32:14/32:16/32:18-32:21/32:23/32:25-32:27N/32:29/32:31/32:33:01/32:35-32:51/32:53-32:65/32:67-32:70/32:72-32:74/32:76-32:78/32:80-32:84	A32(19), -, Null																		18						
A*32:13	-	1																	18						
A*32:15	-																		18	19					

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A*32:17	-				w								12						18						
A*32:24	-		2																18						
A*32:75	-																		w						
A*33:01:01-33:01:04/33:01:06-33:01:08/33:03:01-33:03:25/33:03:28-33:18:01/33:20/33:22-33:29/33:31/33:33-33:34/33:36-33:37/33:39-33:48/33:50-33:52/33:54-33:58/33:60-33:65/33:67-33:80N/33:82-33:88/33:90-33:101/33:103-33:106, B*07:02:40, B*08:01:07, B*15:02:07, B*55:02:10	A33(19), -, A19, Null, B8																			19					
A*33:03:26/33:66	-																			w					
A*33:49	-													13						19					
A*33:59/33:102	-														w					19					
A*34:01:01-34:01:02/34:05/34:11/34:14	A34(10), -									9		11	12												
A*34:02:01-34:04/34:07/34:09-34:10N/34:13	A34(10), -, Null									9				13											
A*34:08	-				4					9				13											
A*34:12	-											11	12												
A*43:01	A43										10														24
A*66:10	-	1								9			12												
A*66:19	-									9			12		14										
A*68:01:28	-															15					20	21			
A*68:49N/68:59N	Null																					w			
A*68:103:01-68:103:02	-													13									21		
A*69:01-69:02	A69(28), -																					21	22		
A*69:03	-														14							21	22		
A*74:01:01-74:03/74:05-74:09/74:11-74:12N/74:14N-74:15/74:16:02-74:20/74:22-74:27	A74(19), -, Null																				20				
A*74:04/74:21	-																			19	20				
A*74:13	-				4								12								20				
A*80:01:01:01-80:01:01:02/80:03	A80, -																								24
B*07:225/07:260	-								w																
B*18:27	-				5							w													
B*51:01:34, C*07:02:27	-																							w	

w = weak, ? = nucleotide sequence information not available for the primer matching sequence