

101.553-06 – including *Taq* pol., IFU-01  
101.553-06u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **99X**

Lot-specific information

## **Olerup SSP® HLA-B\*81**

<b>Product number:</b>	<b>101.553-06 – including <i>Taq</i> pol.</b> <b>101.553-06u – without <i>Taq</i> pol.</b>
<b>Lot number:</b>	<b>99X</b>
<b>Expiry date:</b>	<b>2017-October-01</b>
<b>Number of tests:</b>	<b>6</b>
<b>Number of wells per test:</b>	<b>8+1</b>
<b>Storage - pre-aliquoted primers:</b>	<b>dark at -20°C</b>
- PCR Master Mix:	<b>-20°C</b>
- Adhesive PCR seals	<b>RT</b>
- Product Insert	<b>RT</b>

### **This Product Description is only valid for Lot No. 99X.**

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

### **CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® HLA-B\*81 LOT (08V).**

The HLA-B\*81 kit is updated for new alleles to enable separation of:

- Confirmed<sup>1</sup> 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.

One well has been added to HLA-B\*81, well **9**.

A well containing Negative Control primer pairs has been added.

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

<sup>1</sup>As described in section Uniquely Identified Alleles.

The HLA-B\*81 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B\*81 lot was made (**Lot No. 08V**). The kit design is based on IMGT/HLA database 3.19.0.

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As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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	Added	Added	Updated negative control moved to well 9, primer pair added for the B*81:07 allele.
9	-	-	Updated negative control.

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Well **9** 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
<b>5'-primer<sup>1</sup></b>	<b>164</b>	<b>340</b>	<b>440</b>	<b>45</b>	<b>45</b>	<b>43</b>	<b>36</b>
	5'-CAC <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-TTA <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-TAC <sup>3'</sup>
							<b>36</b>
							5'-TAT <sup>3'</sup>
<b>3'-primer<sup>2</sup></b>	<b>231</b>	<b>2<sup>nd</sup> I</b>	<b>507</b>	<b>59</b>	<b>58</b>	<b>57</b>	<b>47</b>
	5'-TgC <sup>3'</sup>	5'-AAA <sup>3'</sup>	5'-TTg <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-ACA <sup>3'</sup>
							<b>48</b>
							5'-gCA <sup>3'</sup>
							<b>48</b>
							5'-gCC <sup>3'</sup>
							<b>52</b>
							5'-TgT <sup>3'</sup>
<b>A*</b>	<b>+</b>	<b>+</b>	<b>+</b>				
<b>B*</b>	<b>+</b>	<b>+</b>	<b>+</b>				
<b>C*</b>	<b>+</b>	<b>+</b>	<b>+</b>				
<b>DRB1</b>				<b>+</b>	<b>+</b>		
<b>DRB3</b>				<b>+</b>	<b>+</b>		
<b>DRB5</b>				<b>+</b>			
<b>DQB1</b>					<b>+</b>		
<b>DPB1</b>						<b>+</b>	
<b>DQA1</b>							<b>+</b>

<sup>1</sup>The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2<sup>nd</sup> or 3<sup>rd</sup> 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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>2</sup>The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2<sup>nd</sup> or 3<sup>rd</sup> exon or the 2<sup>nd</sup> 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](http://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**

**HLA-B\*81 SSP typing**

**CONTENT**

The primer set contains 5'- and 3'-primers for separating the B\*81:01 to B\*81:07 alleles.

**PLATE LAYOUT**

Each HLA-B\*81 test consists of 9 PCR reactions in a 16 well cut PCR plate. Wells 10 to 16 are empty.

<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
<b>NC</b>	empty	empty	empty	empty	empty	empty	empty

The 16 well PCR plate is marked with 'HLA-B\*81' in silver/gray ink.

Well No. 1 is marked with the Lot No. '99X'.

Wells 1 to 8 – HLA-B\*81 high resolution primers.

Well 9 – 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 heat-sealed with a PCR-compatible foil.

**Please note:** When removing each 16 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**

Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B\*81 alleles will be amplified by primer mixes 1 to 7. In addition, a few HLA-C alleles will be amplified by primer mixes 7.

For further details see Specificity Table.

**UNIQUELY IDENTIFIED ALLELES**

All the HLA-B\*81 alleles, i.e. **B\*81:01 to B\*81:07**, recognized by the HLA Nomenclature Committee in January 2015<sup>1,2,3</sup> will be amplified by the primers in the HLA-B\*81 SSP kit.

The HLA-B\*81 kit enables separation of the confirmed HLA-B\*81 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\*81 alleles is listed below.

The HLA-B\*81 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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<sup>1</sup>HLA-B alleles listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

<sup>2</sup>Alleles 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>.

<sup>3</sup>The B\*81:02 and the B\*07:174, B\*07:202 and 07:222 alleles will give rise to identical amplification patterns. These alleles can e.g. be distinguished by the HLA-B low resolution primer set and/or HLA-B\*07 high resolution kit.

**ALLELE CONFIRMATION STATUS**

Allele	Status <sup>1</sup>
<b>B*81:01</b>	<b>Confirmed</b>
<b>B*81:02</b>	<b>Confirmed</b>
B*81:03	Unconfirmed
B*81:04N	Unconfirmed
<b>B*81:05</b>	<b>Confirmed</b>
B*81:06	Unconfirmed
B*81:07	Unconfirmed

<sup>1</sup>Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

**RESOLUTION IN HOMO- AND HETEROZYGOTES**

Results file with resolution in HLA-B\*81 homo- and heterozygotes is available upon request.

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**SPECIFICITY TABLE**

**HLA-B\*81 SSP subtyping**

Specificities and sizes of the PCR products of the 8+1 primer mixes used for HLA-B\*81 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*81 alleles <sup>3</sup>	Other amplified HLA-B alleles <sup>4</sup>
1	175 bp	800 bp	*81:01-81:07	*07:174, 07:202, 07:222, 27:24, 40:31, 40:45, 40:80, 48:01:01-48:01:06, 48:04, 48:06-48:07, 48:09, 48:11, 48:15-48:16, 48:18-48:20, 48:22, 48:24, 48:27-48:36
2	410 bp	1070 bp	*81:01, 81:03 <sup>2</sup> -81:07 <sup>2</sup>	*07:65, 27:01-27:11, 27:13-27:15, 27:17, 27:19-27:21, 27:24-27:28, 27:30-27:38, 27:40-27:58, 27:60-27:76, 27:78-27:91, 27:93-27:118, 27:120-27:128, 27:130-27:135, 35:76, 35:186, 35:229, 44:90, 44:97, 54:01:01-54:29, 54:31-54:34, 55:01:01-55:05, 55:07-55:17, 55:19-55:70, 56:01:01:01-56:16, 56:18-56:22, 56:24-56:32, 56:34-56:41, 56:43, 56:45, 82:01-82:03, 83:01
3 <sup>6</sup>	405 bp	1070 bp	*81:02, 81:03 <sup>2</sup> -81:07 <sup>2</sup>	*07:02:01-07:02:45, 07:04-07:07, 07:09-07:15, 07:17-07:26, 07:28-07:31, 07:33:01-07:36, 07:39-07:46, 07:48-07:49N, 07:51-07:64, 07:66-07:68:03, 07:70-07:84, 07:86-07:179, 07:181N-07:206, 07:208-07:218, 07:220-07:227, 07:229-07:231N, 07:233-07:241, 08:123, 08:132, 14:21, 38:26, 42:01:01-42:02, 42:04-42:06, 42:08-42:10, 42:12-42:20, 45:06, 67:01:01-67:05, 73:01-73:02
4	285 bp	1070 bp	*81:01-81:07	*07:02:01-07:02:38, 07:02:40-07:06, 07:08-07:11, 07:13, 07:15-07:17, 07:20-07:32, 07:34-07:39, 07:41-07:52, 07:54-07:59, 07:61-07:76, 07:79-07:83, 07:85-07:99, 07:101-07:121, 07:123-07:136:02, 07:138, 07:140-07:161N, 07:164, 07:166-07:192, 07:194-07:195, 07:199-07:213, 07:215-07:224, 07:226, 07:228-07:234, 07:236-07:238, 07:240-07:241, 08:79, 35:66, 37:07, 40:15-40:16, 40:31-40:32, 40:45, 40:80, 40:98, 42:05:01-42:05:02, 48:01:01-48:01:06, 48:05-48:12, 48:14-48:16, 48:18-48:20, 48:22, 48:27-48:36
5 <sup>5,6</sup>	105 bp 165 bp	1070 bp	*81:05 *81:03	*07:83 *15:20, 15:228, 15:305, 35:01:01:01-35:01:27, 35:01:29-35:30, 35:32:01-35:34, 35:36-35:42:02, 35:44-35:45, 35:47-35:56, 35:58-35:59,

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				35:61:01-35:72, 35:74-35:78, 35:80-35:86, 35:88-35:184, 35:186-35:278, 48:02:01-48:02:03, 51:01:01:01-51:24:05, 51:26-51:41N, 51:43-51:46, 51:48-51:156, 51:158:01-51:163, 51:165-51:185, 52:01:01:01-52:06:02, 52:08-52:44, 53:01:01-53:39, 56:05:01-56:05:02, 56:21, 56:36, 58:01:01-58:02, 58:04-58:10N, 58:12-58:19, 58:21-58:29, 58:31N-58:69, 67:05, 78:01:01-78:08, 83:01 <sup>2</sup>	
<b>6<sup>5</sup></b>	80 bp 105 bp	1070 bp	*81:04N *81:05	*07:83	
<b>7</b>	165 bp	1070 bp	*81:06	*07:13, 07:110, 37:52, 38:11, 48:36, 54:02, 67:02, <b>C*03:137, C*03:182, C*07:102<sup>w</sup>, C*07:351, C*12:82</b>	
<b>8</b>	140 bp	1070 bp	*81:07		
<b>9<sup>7</sup></b>	-	-	<b>Negative Control</b>		

<sup>1</sup>Alleles 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-C\*07 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.

<sup>2</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 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.

<sup>3</sup>For several HLA Class I alleles 1<sup>st</sup> and/or 4<sup>th</sup> 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.

<sup>4</sup>Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B\*81 alleles will be amplified by primer mixes 1 to 7. In addition, a few HLA-C alleles will be amplified by primer mix 7.

<sup>5</sup>HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

<sup>6</sup>Primer mixes 3 and 5 may have tendencies of unspecific amplification.

<sup>7</sup>Primer mix 9 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

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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.

‘w’, might be weakly amplified.

‘?’, the nucleotide sequence of the primer matching region is not known.



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**PRIMER SPECIFICATION**

Well No.	1	2	3	4	5	6	7	8
Length of spec. PCR product	175	410	405	285	105	80	165	140
					165	105		
Length of int. pos. control <sup>1</sup>	800	1070	1070	1070	1070	1070	1070	1070
5'-primer(s) <sup>2</sup>	363	41	44	363	206	206	103	363
	5' -AgC 3'	5' -CTg 3'	5' -ggC 3'	5' -AgC 3'	5' -AgA 3'	5' -AgA 3'	5' -CCT 3'	5' -AgC 3'
					652		103	
					5' -CCg 3'		5' -CCT 3'	
3'-primer(s) <sup>3</sup>	499	282	282	605	272	244	226	461
	5' -ggA 3'	5' -gCC 3'	5' -gCC 3'	5' -gCT 3'	5' -TgA 3'	5' -CTA 3'	5' -CAC 3'	5' -gCT 3'
					774	272		
					5' -ggT 3'	5' -TgA 3'		
Well No.	1	2	3	4	5	6	7	8

<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 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.

<sup>2</sup>The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>3</sup>The 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](http://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				HLA-B*81 SSP primer set <sup>2</sup>								
				Well								
				1	2	3	4	5	6	7	8	
				Prod. No.:	201548301	201548302	201548303	201548304	201548305	201548306	201548307	201548308
	IHWC cell line <sup>1</sup>	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 QBL	*18:01		-	-	-	-	-	-	-	-	-
8	9025 DEU	*35:01		-	-	-	-	+	-	-	-	-
9	9026 YAR	*38:01		-	-	-	-	-	-	-	-	-
10	9107 LKT3	*54:01		-	+	-	-	-	-	-	-	-
11	9051 PITOUT	*44:03		-	-	-	-	-	-	-	-	-
12	9052 DBB	*57:01		-	-	-	-	-	-	-	-	-
13	9025 JESTHOM	*27:05		-	+	-	-	-	-	-	-	-
14	9071 OLGA	*15:01	*15:20	-	-	-	-	+	-	-	-	-
15	9075 DKB	*40:01		-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*40:02		-	-	-	-	-	-	-	-	-
17	9282 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	9320 BEL5GB	*44:02	*44:03	-	-	-	-	-	-	-	-	-
29	9050 MOU	*44:03		-	-	-	-	-	-	-	-	-
30	9021 RSH	*42:01		-	-	+	-	-	-	-	-	-
31	9019 DUCAF	*18:01		-	-	-	-	-	-	-	-	-
32	9297 HAG	*41:02		-	-	-	-	-	-	-	-	-
33	9098 MT14B	*40:01		-	-	-	-	-	-	-	-	-
34	9104 DHIF	*38:01		-	-	-	-	-	-	-	-	-
35	9302 SSTO	*44:02		-	-	-	-	-	-	-	-	-
36	9024 KT17	*15:01	*35:01	-	-	-	-	+	-	-	-	-
37	9065 HHKB	*07:02		-	-	+	+	-	-	-	-	-
38	9099 LZL	*15:01		-	-	-	-	-	-	-	-	-
39	9315 CML	*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	9239 SHJO	*42:01	*50:01	-	-	+	-	-	-	-	-	-
46	9013 SCHU	*07:02		-	-	+	+	-	-	-	-	-
47	9045 TUBO	*51:01		-	-	-	-	+	-	-	-	-
48	9303 TER-ND	*35:01	*44:03	-	-	-	-	+	-	-	-	-

<sup>1</sup>The 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.

101.553-06 – including *Taq* pol., IFU-01  
101.553-06u – without *Taq* pol., IFU-02

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Lot No.: **99X**

#### Lot-specific information

<sup>2</sup>The 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.

No DNAs carrying the alleles to be amplified by primer solutions 6, 7 and 8 were available. The specificity of the primers in primer solution 7 were tested by separately adding one additional 5'-primer, respectively two additional 3'-primers. In primer solutions 6 and 8 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. One 3'-primer in primer solution 5 was not possible to test. In addition, one 5'-primer in primer solution 5 was tested by separately adding an additional 3'-primer.

101.553-06 – including *Taq* pol., IFU-01  
101.553-06u – without *Taq* pol., IFU-02

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Lot No.: **99X**

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