

# QIAGEN Supplementary Protocol

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## Selection, ordering, and use of HaploPrep HLA locus probes for automated extraction of HLA haplotypes using the EZ1<sup>®</sup> HaploPrep Kit

The QIAGEN<sup>®</sup> EZ1 HaploPrep Kit uses haplotype-specific extraction (HSE) to physically separate diploid genomic DNA into its haploid components, based on allele distinguishing single nucleotide polymorphisms (SNPs). By separating the alleles, the EZ1 HaploPrep Kit enables individual analysis of each allele by routine DNA typing methods that are currently used on standard diploid DNA. This provides a simple method for resolution of, for example, ambiguous human leukocyte antigen (HLA) typing results caused by new alleles.

This protocol provides information on the selection, sequence, ordering, and use of HLA locus-specific HaploPrep probes for physical separation of all currently known HLA-A, -B, -C, -DR, and -DQB1 alleles.

**IMPORTANT:** Please read the handbook supplied with the EZ1 HaploPrep Kit, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure. The EZ1 HaploPrep Kit is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products.

## Procedure

### Selecting HaploPrep HLA locus probes

#### 1. Select the probe.

- Identify the low-resolution HLA type of your sample using a routine HLA typing method, and select a suitable HaploPrep HLA locus probe from the probe selection books available at the QIAGEN Web site ([www.qiagen.com/goto/HaploPrepProbes](http://www.qiagen.com/goto/HaploPrepProbes)).
- Alternatively, identify heterozygous positions in diploid DNA at the cDNA nucleotide positions covered by the HaploPrep HLA locus probes, and select the probe for a specific allele. (The nomenclature for HaploPrep HLA locus probes is as follows. “A299C”, as an example, means HLA-A locus, cDNA nucleotide position 299, and the nucleotide on the allele extracted with this probe is cytosine.)



## 2. Select the sequence information.

- Select the sequence information for the appropriate probe from the following tables.

**Table 1. HaploPrep HLA-A locus probes**

<b>Probe name</b>	<b>Oligonucleotide sequence</b>
A257A	CAT ATT CCG TGT CTC CTG
A259A	CTT CAC ATT CCG TGT GTT C
A299C	GGT CCC CAG GTT CGC
A299T	GGT CCC CAG GTC CAC
A314C	TGC GGA TCG CGC TCC
A362G	CAC ACC ITC CAG AGG A*
A362T	TCT CAC ACC ATC CAG ATA
A414C	GCG GGT ACC ACC AGT A
A414G	CGG GTA CCG GCA GG
A538T	TGT GGC GGA GCA GTT G
A559C	CTA CCT GGA GGG CCG
A571G	GTA TCT GCG GAG CCC G

\* I: inosine.

**Table 2. HaploPrep HLA-B locus probes**

<b>Probe name</b>	<b>Oligonucleotide sequence</b>
B48A	CTG CTC TGG GGG GCA G
B48C	CTG CTC TCG GCG GCC C
B261C	GTA TTG GGA CCG GAA C
B261G	GTA TTG GGA CCG GGA G
B277A	CAG ATC TCC AAG ACC AAC A
B277G2	CAG ATC TAC AAG GCC CAG G
B317T	CTG CGG AAC CTG CGC G
B355A	CCG GGT CTC ACA TCA TC
B355C	CCG GGT CTC ACA CCC TC
B355T	CCG GGT CTC ACA CTT GG
B539A	TGT GGC GGA GCA GGA C
B539T	TGT GGC GGA GCA GCT G
B559C	CCT ACC TGG AGG GCC T
B559G	CCT ACC TGG AGG GCG A

**Table 3. HaploPrep HLA-C locus probes**

<b>Probe name</b>	<b>Oligonucleotide sequence</b>
Cw97G	CTC CAT GAG GTA TTT CGA
Cw102C	GCC GGG ACA CGG CGG
Cw103T	GTA TTT CTC CAC ATC C
Cw142G	AGC CCC GCT TCA TCG C
Cw176G	CGT CGC TGT CGA ACC G
Cw302A	GCG CAG TTT CCI CAG GTT*
Cw312C	GAG TGA +GC CTG CGG AA+C C <sup>†</sup>
Cw355A	GCC GGG TCT CAC ATC AT
Cw419C	GCG GGT ATG ACC AGT C
Cw419T	GCG GGT ATA ACC AGT T
Cw486C	ACC GCC GCI GAC A+CC* <sup>‡</sup>
Cw486G	ACC GCC GCG GAC ACG
Cw539G	GTG AGG CGG AIC AGT G*
Cw559C	AGC CAC TCC ACG CAC AGG
Cw559G	AGC CAC TCC ACG CAC TCG

\* I: inosine.

<sup>†</sup> +G: LNA modified G.

<sup>‡</sup> +C: LNA modified C.

**Table 4. HaploPrep HLA-DR locus probes**

<b>Probe name</b>	<b>Oligonucleotide sequence</b>
DR28G	ACG TTT CTT GGA GGA GG
DR30C	CGT TTC TTG GAG TAC TCT A
DR37C	CTT GGA GCA GGT TAA ACA
DR37G	CTT GGA GTA CTC TAC GGG
DR38T2	TCT TGA AGC AGG ATA AGT T
DR39G	CTG TGG CAG CCT AAG AGG
DR40A	TGT GGC AGG GTA AGT ATA
DR42A	GTG GCA GCT TAA GTT TGA A
DR140T	CCG TCA CCG CCC GGA
DR170G	GTT CCA GTA CTC GGC GCT
DR173A	GGC TGT TCC AGT ACT CCT
DR174G	GTT CCA GTG CTC CGC AG
DR210C	GAC ATC CTG GAA GAC GA
DR220C	CAG TAG GTG TCC ACC AG

**Table 5. HaploPrep HLA-DQB1 locus probes**

Probe name	Oligonucleotide sequence
DQB1-122T	CAG ATG CCC TTA AAC TGG +AA*
DQB1-173A	GGA GCG CGT GCG TTA T
DQB1-173G	CGA GCG CGT GCG IGG T†
DQB1-173T	AGA GCG CGT GCG TCT T
DQB1-185G	CGT CTT GTG AGC AGA AGC
DQB1-266T	GCT GTT CCA GTA CTC GIC AAC‡
DQB1-267T	CAG IIG CGG CCT GAT G†
DQB1-304G	GAA GGA AGT CCT GGA G+GG‡
DQB1-319T	GGA CCC GGG CGG AG+T§
DQB1-326C	CGG GCG GAG TTG GAC AC
DQB1-353C	AGA CAC AAC TAC GAG GTG G+C¶
DQB1-356T	CAC AAC TAC CAI TTG GAG CTC†

\* +A: LNA modified A.

† I: inosine.

‡ +G: LNA modified G.

§ +T: LNA modified T.

¶ +C: LNA modified C.

## Ordering HaploPrep HLA locus probes

### 3. Order the probes.

- Order oligonucleotides in salt-free quality (e.g., from IDT in LabReady format, i.e., adjusted to 100  $\mu$ M with TE).\*\*
- LNA modified oligonucleotides are provided by Exiqon.\*\*
- No biotin modification of probes is required.

\*\*This is not a complete list of suppliers and does not include many important vendors of biological supplies.

## Using HaploPrep HLA locus probes

### 4. Use the probes following the EZ1 HaploPrep protocol.

- Use probes at 100  $\mu$ M concentration according to the EZ1 HaploPrep procedure, and follow the protocol as described in the *EZ1 HaploPrep Handbook*.

## Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the EZ1 HaploPrep Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at [www.qiagen.com/Support](http://www.qiagen.com/Support) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

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Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from [www.qiagen.com/Support/MSDS.aspx](http://www.qiagen.com/Support/MSDS.aspx).

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HSE technology is developed under a collaboration of QIAGEN and Generation Biotech. It is covered by EP patent 00984206.3 and its foreign counterparts.

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