Mentype® **DigitalScreen**

Instructions for Use

For research use only

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Made in Germany

Biotype GmbH develops, produces, and distributes PCR-based applications for medical diagnostics.

Our Mentype® test kits guarantee highest quality standards.

We are at your disposal for further information and suggestions.
Contact us or visit our website www.biotype.de.

Content

Mentype® **DigitalScreen**

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1. Product description

1.1 Intended Use

The Mentype® **DigitalScreen** test kit is designed to determine the allele distribution of insertion-deletion polymorphisms in unmixed DNA samples qualitatively. A quantification using the Mentype® **DigitalScreen** kit is not possible.

The test kit is only intended to be used for research purposes, a use for diagnostic purposes is not permitted.

The test kit should only be used by professional users trained in molecular biological techniques in general and in performing digital PCR in particular

1.2 Overview and Explanation

The Mentype® **Digital** approach deploys the highly sensitive digital PCR technology that allows absolute quantification of chimerism samples. Specific for digital PCR is the sample-partitioning into a plethora of nano droplets. Each droplet represents a separate compartment containing nano liters of the sample of interest. During thermal cycling each compartment functions as a separate PCR amplification chamber. Depending on how many copies of the target DNA molecules have been dispensed into the droplets (zero, one or more copies) a multitude of replicates is generated per single PCR run. Using Poisson statistics, the absolute number of starting copies can be determined very accurately. After thermal cycling each droplet is automatically analyzed and determined as positive or negative fraction. Because the digital PCR uses end-point detection of the amplification product, efficiency of the amplification is much less of a concern and calibration curves are not necessary.

Mentype® **DigitalScreen** represent a screening plate and allows the fast identification of biallelic short insertion/deletion polymorphisms (INDELs). The thereby identified markers can be used for subsequent DNA quantification with the kit Mentype® **DigitalQuant**.

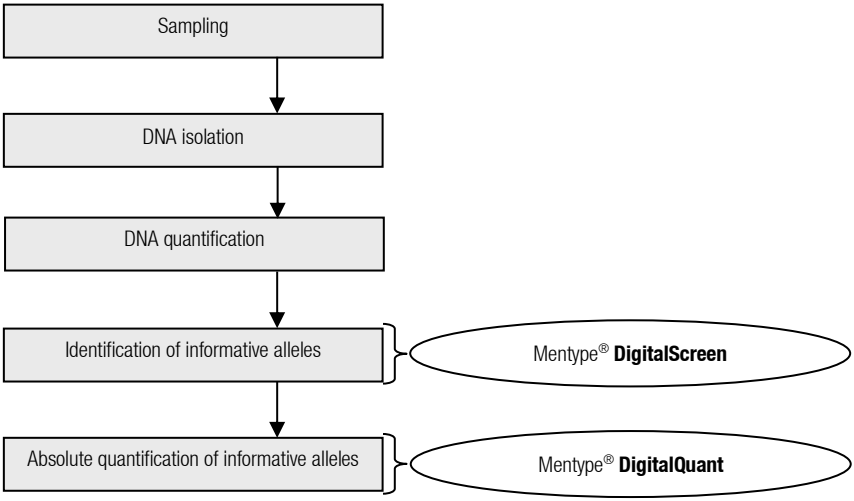

Figure 1 From sample to analysis with Mentype® **DigitalScreen**

All specific DIP markers (see Table 8) are labeled with FAM, the active reference (REF) is labeled with HEX.

1.3 Platform and Software

The test kit was validated using the Bio-Rad QX100™ and QX200™ Droplet Digital™ PCR System and the following thermocycler:

- Applied Biosystem GeneAmp® PCR System 9700 Aluminium and GeneAmp® PCR System 9700 Silber
- Eppendorf Mastercycler ep-S und Mastercycler nexus
- Biometra T1
- Bio-Rad DNA Engine PTC-200.

Running Mentype® **DigitalScreen** assay on instruments not stated above is on one´s own responsibility.

2. Supplied materials

2.1 Kit content

The following reagents for running the Mentype® **DigitalScreen** kit are included:

Table 1 Kit content of a Mentype® **DigitalScreen** kit

Components	Amount	Vol./Tube [µL]	Lid Color	Storage
Nuclease-free Water	2	1,500	Blue	-25 °C to -15 °C
Screening plate Mentype® **DigitalScreen**	4	-	-	4 °C

2.2 Ordering information

Please direct orders in writing to:

Email: sales@biotype.de **Fax:** +49 (0)351 8838 403

Table 2 Ordering Information Mentype® **DigitalScreen** Kits

Product Name	Package Size	Order Nummer
Mentype® **DigitalScreen**	4 Plates	45-64610-0004

2.3 Additional required reagents and consumables

Additional reagents are required to run the Mentype® **DigitalScreen** assay on the ddPCR platform QX100™/QX200™:

Table 3 Additional required reagents for Mentype® **DigitalScreen**

Reagent	Supplier
DIP Positive Control (DPC) (Order no.: 00-10030-0100)	Biotype GmbH
FastDigest *EcoRI* (e. g. Thermo Scientific™)	e. g. Fisher Scientific GmbH
2x ddPCR™ Supermix for Probes (No dUTP)	Bio-Rad Laboratories

Table 4 Standard reagents and devices of the QX200™/QX100™ System

Reagent	Supplier
QX100™/QX200™ Droplet Generator	Bio-Rad Laboratories
PX1 PCR Plate Sealer	Bio-Rad Laboratories
QX100™/QX200™ Droplet Digital™ PCR System	Bio-Rad Laboratories
Droplet Generation Oil for Probes	Bio-Rad Laboratories
ddPCR™ Droplet Reader Oil	Bio-Rad Laboratories
DG8™ Cartridge Holder	Bio-Rad Laboratories
DG8™ Cartridges for QX200™/QX100™ Droplet Generator	Bio-Rad Laboratories
DG8™ Gaskets for QX200™/QX100™ Droplet Generator	Bio-Rad Laboratories
PCR Plate Heat Seal, foil, pierceable	Bio-Rad Laboratories
ddPCR™ 96-Well Plates	Bio-Rad Laboratories
96-well PCR foil	Any

3. Warning and safety instructions

3.1 Warnings and safety instructions

Please note the safety data sheet

Please contact the respective manufacturers for copies of the SDS for any additionally needed reagents.

3.2 Quality assurance

All kit components undergo an intensive quality assurance process at Biotype GmbH. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance (support@biotype.de).

3.3 Trademarks and patents

Mentype® is a registered trademark of Biotype GmbH.

3.4 Storage

The Mentype® **DigitalScreen** kit must be stored at a temperature of 4 °C (refrigerator) protected from light and kept dry.

The expiry date of the kits is indicated on the outer label.

4. Mentype® DigitalScreen

Analysis of DNA ratios starts with Mentype® **DigitalScreen** to determine which Mentype® **DigitalQuant** assays can be applied for quantification.
The Mentype® **DigitalScreen** plate contains dehydrated locus-specific primer mixes and the active reference (REF). Two DNA samples (recipient and donor) can be screened against the panel of 30 assays in one run. The layout is shown below (Figure 2).
Take care to **use the plate in the right direction**, with the letters at the left-hand site.

	1	2	3	4	5	6	7	8	9	10	11	12
A	67-D	104-D	131-I	301-I	67-D	104-D	131-I	301-I				
B	70-D	104-I	133-I	304-D	70-D	104-I	133-I	304-D				
C	70-I	105-D	134-I	304-I	70-I	105-D	134-I	304-I				
D	88-D	105-I	140-I	307-I	88-D	105-I	140-I	307-I				
E	88-I	106-I	152-D	310-I	88-I	106-I	152-D	310-I				
F	97-I	114-D	163-D	SRY	97-I	114-D	163-D	SRY				
G	101-D	114-I	163-I	PTC	101-D	114-I	163-I	PTC				
H	101-I	128-D	301-D	NTC	101-I	128-D	301-D	NTC				

DNA 1 DNA 2

Figure 2 Plate layout for Mentype® **DigitalScreen**

4.1 Sampling and handling

This test is intended for the use of DNA extracted from peripheral blood samples as well as bone marrow. The use of other samples (e. g. sorted cells) must be independently validated by the user.

For DNA extraction, commercially available kits for isolating of genomic DNA, e. g. by the provider Qiagen or Machery-Nagel, should be used.

Concentrated DNA can be adjusted to the required concentration by dilution with 1x TE buffer. DNA should be stored at -20 °C ± 5 °C.

4.2 PCR Mastermix preparation

Table 5 (below) shows required volumes and the final concentration of the reagents. Column 4 depicts an example of how to prepare the PCR-Master mix for screening with one plate. A sample volume of 5 µL is shown.

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the PCR-Master mix. Add one or two reactions to the truly required reaction-number to compensate pipetting errors. Dispense the PCR-Master mix equally to the wells. The volume depends on the applied DNA sample volume.

Table 5 Reaction procedure for processing a Mentype® **DigitalScreen** plate using 5 µL DNA per well

Component	Volume per Reaction	Final Concentration	Master mix for 66 reactions (1 plate)
2x ddPCR™ Supermix for Probes (No dUTP)	10.0 µL	1 x	660.0 µL
FastDigest *EcoRI* enzyme	0.5 µL		33.0 µL
Nuclease-free Water	4.5 µL		297.0 µL
Volume mastermix/well	15.0 µL		15.0 µL
DNA sample (2-4 ng/µL)	5.0 µL	10-20 ng/well	5.0 µL/well

Note: The minimum DNA concentration for each sample per well should be **10 ng-20 ng** in a maximum volume of 9.5 µL. All wells for a given sample have to contain the same amount of DNA.

Prepare 15 µL of the master mix in each well, then pipette 5 µL of the appropriate DNA or control into each well. Seal the plate with a PCR foil and mix the plate thoroughly (vortexing), so that the dried primers completely dissolve. Centrifuge the plate briefly.

4.2.1. Positive control PTC (not included)

Apply 5 µL of DIP Positive Control DNA mix (DPC, ready for use) instead of template DNA. Pipette the GPC into the second-last well of column 4 (**G4**) and column 8 (**G8**) of the 96-well screening plate. The wells contain the reference assays (see Figure 2).

4.2.2. No Template control NTC

Nuclease-free water serves as no template control (NTC). Instead of DNA template, pipette respective volume into well **H4 and H8** of the 96-well screening plate. The wells also contain the reference assays (see Figure 2).

4.3 Restriction Digestion

The Biotype Mentype® **Digital** assays are specific for *EcoRI* restriction digestion.

Restriction digestion of the DNA to be analyzed prior to droplet generation is recommended. The digestion can be carried out directly in the PCR reaction vessel. Use max. 1 µL of FastDigest *EcoRI* enzyme (see Table 5) to digest up to 1 µg of genomic DNA in a total volume of 20 µL. For the use of Non-FastDigest *EcoRI* enzyme 2 units per 20 µl reaction are recommended.

After preparing the Screening Plate with PCR Master Mix, sample DNA and controls, cover the plate with a PCR tube (not supplied), mix thoroughly and centrifuge briefly. Then incubate the screening plate in a thermocycler for **10 min at 37 °C** for restriction digestion.

Note: The restriction digestion of DNA amounts >100 ng per well must be performed in an extra step and before the start of the PCR.

4.4 Droplet Generation using the QX100/QX200 droplet generator

For optimal results freshly mix samples dispensed in the Mentype® **DigitalScreen** plate before droplet generation. Place the DG8 cartridge into the cartridge holder. Pipette each sample (20 µL of the digested PCR mix) up and down for 3 times before transferring the sample to the sample-wells of the DG8 cartridge (see Figure 4, middle row; also see the general guidelines from Bio-Rad for droplet generation).

Start with pipetting row 1A – 1H of the digested PCR mix. Transfer samples in the DG8 cartridge from left to right. After transferring all 8 samples, fill 70 µL of Droplet Generation (DG) Oil in the bottom-line wells. Hook the gasket over the cartridge holder by using the holes on both sides.

All 8 sample wells in the DG8 cartridge must be filled either with sample or 1x Biorad “Buffer Control” (not provided). All 8 oil wells have to contain DG oil.

(Always use the holder to load the DG8 cartridge. Do not dispense the DG oil before all wells are prepared with sample).

Note: When filling the DG8 Cartridge, always use the holder provided. The DG oil must first be distributed into the 8 wells / reaction vessels of the DG8 cartridge when all 8 wells / reaction vessels have been filled with sample.

Place the filled DG8 cartridge into the QX100/QX200 droplet generator.

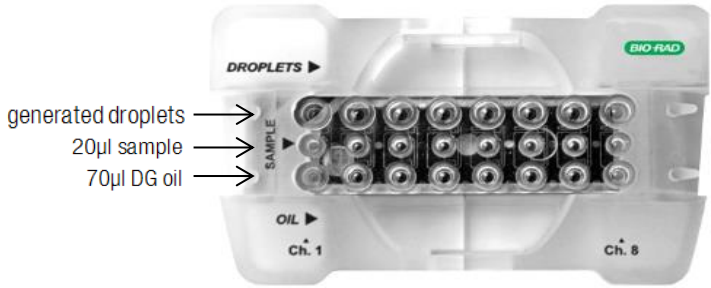

Figure 3 Cartridge holder with clamped cartridge, the label shows the position of PCR-approach (20 µL sample), Droplet Generation Oil (70 µL DG oil) and processed droplets

After droplet generation, the top wells of the cartridge contain droplet-samples. Transfer 40 µL of the droplet-samples into a 96-well PCR plate.

Tip: Use an 8-channel pipette to save time).

Proceed the same way with samples of row 2A – 2H to 8A – 8H.

Note: Upon droplet generation handle sample gently (no vortex, no spin-down).

Seal the 96-well PCR plate with Pierceable Foil Heat Seal and place the plate into the PX1 PCR Plate Sealer. Also see the Bio-Rad instructions in the PX1 PCR Plate Sealer Instruction Manual.

4.5 Digital PCR amplification

When heat sealing is completed, place the 96-well PCR plate into a thermal cycler and start the program according to Table 6.

Note: Do not vortex or/and spin down the plate.

Table 6 Amplification parameters* of the Mentype® **DigitalScreen**

Temperature	Time	Cycles	Ramp Rate**
95 °C	10 min	1x	2 °C/s
94 °C	30 s	40x	
62 °C	60 s		
98 °C	10 min	1x	
4 °C	∞	1x	1 °C/s

*use a heated lid and set to 105 °C. Set sample volume to 40 µL
**ramp rate depends on the PCR cycler and the block material:

> For PCR cycler with **aluminium block** use a ramp rate of **2 °C/s**.

> For PCR cycler with **silver block** use **1 °C/s;**

> If you **cannot determine the block-material** use a ramp rate of **1 °C/s.**

4.6 Read droplets and analyze results

After the thermal cycling is finished, place the PCR plate containing the amplified sample-droplets into the holder of the QX100/QX200 droplet reader.

Note: Do not vortex and/or spin down the plate after cycling. Also see the instrument-specific guidelines.

4.6.1. Create the plate layout

Open the software Quanta™Soft from Bio-Rad. Create the plate layout for your experiment (see Figure 2). Open the editor (Applied Well Settings) by double-clicking on a well in the plate layout. Assign the sample name, the type of experiment, and determine which assay corresponds to which fluorescence channel. Then you assign the sample names of the recipient and donor DNA.

For Mentype® **DigitalScreen,** please define the settings according to Table 7.

Table 7 Settings to be determined for analyzing the Mentype® **DigitalScreen** Kit in the Quanta™Soft

Sample and experiment types	Settings
Sample	
Name	give a name
Experiment	Absolute Quantification (ABS)
Supermix	ddPCR Supermix for Probes (no dUTP)
Target 1	
Name	Marker name e.g. DP67
Type	e.g. Ch 1 Unknown
Target 2	
Name	REF
Type	e.g. Ch 2 Unknown

Note: All specific DIP-Markers (see Table 9) are labeled with FAM. The reference (REF) is labelled with HEX accordingly.

After definition of the experiment click **Run**.

The droplet reader counts fluorescence-positive and negative droplets for absolute quantification of target DNA. Each sample-containing droplet is individually processed and verified for both FAM and HEX fluorescence. Data from at **least 10,000** accepted droplets are used for the concentration calculations.

4.6.2. Data analysis

Load the plate in the Setup window of the Quanta™Soft Software (Bio-Rad). Click **Analyze** to open and analyze the data. Review the data in the 2D Amplitude channel to verify whether the automated threshold and the cluster separation is correct (see Figure 4).

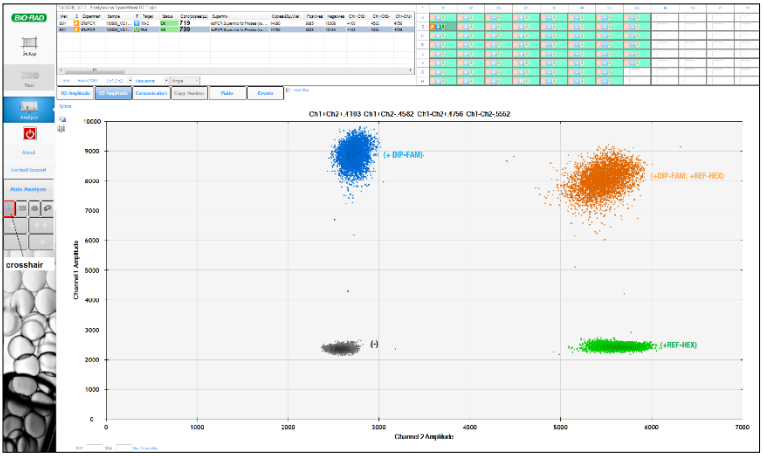

Figure 4 2D amplitude view (scatter plot) of the droplet fluorescence

All 4 cluster (grey, green, brown, blue) need to appear fully separated (see Figure 4). If clusters are not accurately separated or the automated threshold is not correct you have to draw corrections manually. Therefore, use the threshold adjustment tools (crosshair).

The droplets are interpreted as follows: double negative (gray), FAM positive (blue), HEX positive (green) and double positive (orange - positive for FAM and HEX in the same droplet).

Manual correction was successful when the color of the cluster or individual droplets changes and the crosshairs are displayed in pink.

Then select the wells to analyze and click on **Table.** Then open the **Result Table** to see the results.

The detection limit for a successful experiment is five FAM-positive droplets. A result with less than five droplets is defined as negative, the FAM cluster was not recognized.

4.6.3. Identification of informative loci

Review results and compare FAM cluster, outlining DIP loci, between the initial samples to identify specific loci. DNA 1 positive loci, which are negative in DNA 2 sample, are

recommended to be used for the absolute quantification and analysis with Mentype® **DigitalQuant** duplex assays. DNA 2 positive loci, that are negative in DNA 1 sample, can however likewise be used to analyse mixes samples.

To determine if the identified DIP allele (AOI) is homozygous or heterozygous, use the following formula:

Ratio in percent = (100 * conc AOI) / conc REF

If the percentage of copies/µL of AOI to copies/µL of reference (REF) is **less than 65%**, the AOI in the marker is **heterozygous**. If the ratio is **greater than 65%**, the marker is **homozygous**. This information must be taken into account when calculating the quantification with Mentype® **DigitalQuant** (see IFU Mentype® **DigitalQuant**).

For a statistically reliable and robust DNA analysis, the analysis of at least 2 and optimally 3 informative loci is recommended. After selecting the specific loci, you can order the corresponding Mentype® **DigitalQuant** assays (see Table 10, Table 11) using the following contact details:

Email: sales@biotype.de **Fax:** +49 (0) 351 8838 403.

5. Characteristics and availability of Mentype® DigitalQuant assays

Table 8 Locus-specific information and chromosomal location

Locus	Chromosomal Location
67	5q33.3
70	6q16.1
88	9q22.33
97	13q13.1
101	15q26.1
104	13q32.1
105	14q24.3
106	16q13
114	17p13.2
128	1q31.3
131	7q36.2
133	3p22.1
134	5q11.2
140	3q23
152	16p13.2
163	12q24.31
301	17q21.32
304	9q34.3
307	Xp11.23
310	2p22.3
SRY	Yp11.2

Table 9 Available Mentype® **DigitalQuant** assays

Loci	Deletion (- Allele)	Insertion (+ Allele)	Allele specific duplex assay with Reference (REF)	Allele-specific Duplex-Assay with Marker for Y-chromosomal region (SRY)
67	67-D		DP67-D+REF	DP67-D+SRY
70	70-D		DP70-D+REF	DP70-D+SRY
		70-I	DP70-I+REF	DP70-I+SRY
88	88-D		DP88-D+REF	DP88-D+SRY
		88-I	DP88-I+REF	DP88-I+SRY
97		97-I	DP97-I+REF	DP97-I+SRY
101	101-D		DP101-D+REF	DP101-D+SRY
		101-I	DP101-I+REF	DP101-I+SRY
104	104-D		DP104-D+REF	DP104-D+SRY
		104-I	DP104-I+REF	DP104-I+SRY
105	105-D		DP105-D+REF	DP105-D+SRY
		105-I	DP105-I+REF	DP105-I+SRY
106		106-I	DP106-I+REF	DP106-I+SRY
114	114-D		DP114-D+REF	DP114-D+SRY
		114-I	DP114-I+REF	DP114-I+SRY
128	128-D		DP128-D+REF	DP128-D+SRY
131		131-I	DP131-I+REF	DP131-I+SRY
133		133-I	DP133-I+REF	DP133-I+SRY
134		134-I	DP134-I+REF	DP134-I+SRY
140		140-I	DP140-I+REF	DP140-I+SRY
152	152-D		DP152-D+REF	DP152-D+SRY
163	163-D		DP163-D+REF	DP163-D+SRY
		163-I	DP163-I+REF	DP163-I+SRY
301	301-D		DP301-D+REF	DP301-D+SRY
		301-I	DP301-I+REF	DP301-I+SRY
304	304-D		DP304-D+REF	DP304-D+SRY
		304-I	DP304-I+REF	DP304-I+SRY
307		307-I	DP307-I+REF	DP307-I+SRY
310		310-I	DP310-I+REF	DP310-I+SRY
SRY		SRY	DPSRY+REF	

6. References

Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, Lamy T, Le Prise PY, Beauplet A, Bories D, Semana G, Quelvennec E. (2002) Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood 99, 4618-4625.

Vogelstein B, Kinzler KW (1999) Digital PCR. Proc Natl Acad Sci USA96:9236-9241.

George D, Czech J, John B, Yu M, Jennings LJ (2013) Detection and quantification of chimerism by droplet digital PCR. Chimerism, 4:102-108.

Manoj P (2014) Droplet digital PCR technology promises new applications and research areas. Mitochondrial DNA 2014, e-published ahead of print [doi: 10.3109/19401736.2014.913168].

Jim F. Huggett, Carole A. Foy, Vladimir Benes, Kerry Emslie,Jeremy A. Garson, Ross Haynes, Jan Hellemans, Mikael Kubista, Reinhold D. Mueller, Tania Nolan, Michael W. Pfaffl,Gregory L. Shipley, Jo Vandesompele, Carl T. Wittwer, and Stephen A. Bustin13 (2013) Guidelines for Minimum Information for Publication of Quantitative Digital PCR Experiments. Clinical Chemistry 2013.206375.

7. Ordering Information Mentype® DigitalQuant

Table 10 Ordering information for allele specific Mentype® **DigitalQuant** assays – these assays are available to you anytime (warehousing)

Assay	25 Reactions
DP67-D+REF	45-02011-0025
DP70-D+REF	45-02021-0025
DP70-I+REF	45-02031-0025
DP88-D+REF	45-02041-0025
DP88-I+REF	45-02051-0025
DP97-I+REF	45-02061-0025
DP101-D+REF	45-02071-0025
DP101-I+REF	45-02081-0025
DP104-D+REF	45-02091-0025
DP104-I+REF	45-02101-0025
DP105-D+REF	45-02111-0025
DP105-I+REF	45-02121-0025
DP106-I+REF	45-02131-0025
DP114-D+REF	45-02141-0025
DP114-I+REF	45-02151-0025
DP128-D+REF	45-02161-0025
DP131-I+REF	45-02171-0025
DP133-I+REF	45-02181-0025
DP134-I+REF	45-02191-0025
DP140-I+REF	45-02201-0025
DP152-D+REF	45-02211-0025
DP163-D+REF	45-02221-0025
DP163-I+REF	45-02231-0025
DP301-D+REF	45-02241-0025
DP301-I+REF	45-02251-0025
DP304-D+REF	45-02261-0025
DP304-I+REF	45-02271-0025
DP307-I+REF	45-02281-0025
DP310-I+REF	45-02291-0025
DPSRY+REF	45-02301-0025

Table 11 Ordering information for the allele-specific Mentype® **DigitalQuant** Assays, these assays are made for you upon request (on-demand ordering)

Assay	25 Reactions
DP67-D+SRY	45-02311-0025
DP70-D+SRY	45-02321-0025
DP70-I+SRY	45-02331-0025
DP88-D+SRY	45-02341-0025
DP88-I+SRY	45-02351-0025
DP97-I+SRY	45-02361-0025
DP101-D+SRY	45-02371-0025
DP101-I+SRY	45-02381-0025
DP104-D+SRY	45-02391-0025
DP104-I+SRY	45-02401-0025
DP105-D+SRY	45-02411-0025
DP105-I+SRY	45-02421-0025
DP106-I+SRY	45-02431-0025
DP114-D+SRY	45-02441-0025
DP114-I+SRY	45-02451-0025
DP128-D+SRY	45-02461-0025
DP131-I+SRY	45-02471-0025
DP133-I+SRY	45-02481-0025
DP134-I+SRY	45-02491-0025
DP140-I+SRY	45-02501-0025
DP152-D+SRY	45-02511-0025
DP163-D+SRY	45-02521-0025
DP163-I+SRY	45-02531-0025
DP301-D+SRY	45-02541-0025
DP301-I+SRY	45-02551-0025
DP304-D+SRY	45-02561-0025
DP304-I+SRY	45-02571-0025
DP307-I+SRY	45-02581-0025
DP310-I+SRY	45-02591-0025

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