Mentype®
DIPquant qPCR
Manual

Highly sensitive and accurate quantification of chimerism

For research use only

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Diagnostic GmbH

Made in Germany

Biotype Diagnostic GmbH develops, produces and markets their PCR-based rapid Mentype® Detection Kits. Our products provide customers with fast and reliable testing methods for professional investigation.

Benchmark of Progression

For information and enquiries about the Mentype® **DIPquant** please do not hesitate to get in touch or visit www.biotype.de/en/home.html.

Mentype® **DIPquant** qPCR

Product description

Mentype® **DIPquant** assays mediate flexible and highly quantitative real-time PCR based chimerism monitoring deploying insertion/deletion DNA-polymorphisms (DIPs/INDELs).

Analysis of molecular chimerism resulting from allogeneic stem cell transplantation has become a well established method to control the course of transplant engraftment and to assess the risk of threatening relapse. Molecular chimerism analysis can be performed on diverse DNA-sequence motifs of which biallelic short insertion/deletion polymorphisms are best suited for allele-specific quantitative analyses.

Subsequent to the identification of informative DIP loci performed with the Mentype® **DIPscreen** application, Mentype® **DIPquant** assays provide the quantitative approach. Through allele-specific qPCR monoplex reactions, recipient-specific DIP alleles can be monitored. The flexible assay format thereby allows a sample throughput of high or small quantities at any time required with only minimal amount of consumables spend.

Allele-specific Mentype® **DIPquant** qPCR assays are available for 55 DIP alleles as well as for two Y-chromosome specific regions. ß-Globin serves as active reference (REF) for relative quantification.
All assays were designed running with the same qPCR parameters to allow parallel analysis of multiple DIP-markers. Analysis of several allele-specific DIP assays can be performed at one time in one run.

The test kit was validated by using the Roche LC480, ABI Prism ® 7000 and Qiagen Rotor-Gene. Development, manufacture and distribution of Biotype® products are certified according to DIN EN ISO 9001:2008.

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1. Description of the Mentype® DIPquant

Table 1. Available allele-specific Mentype® DIPquant qPCR assays

DIPscreen Loci	**DIPquant allele-specific qPCR assay**		**DIPscreen Loci**	**DIPquant allele-specific qPCR assay**	
	Deletion (- Allel)	Insertion (+ Allel)		Deletion (- Allel)	Insertion (+ Allel)
FAM Panel			**BTY Panel**		
AM X			HLD48		HLD48-I
AM Y		SRY / SMCY	HLD114	HLD114-D	HLD114-I
HLD106	HLD106-D	HLD106-I	HLD304	HLD304-D	
HLD70	HLD70-D	HLD70-I	HLD131	HLD131-D	HLD131-I
HLD84	HLD84-D	HLD84-I	HLD38		HLD38-I
HLD103	HLD103-D	HLD103-I	HLD82	HLD82-D	HLD82-I
HLD104	HLD104-D	HLD104-I			
HLD116	HLD116-D	HLD116-I	**Active Reference**		Reference
HLD112		HLD112-I			
HLD307	HLD307-D	HLD307-I			
HLD110		HLD110-I			
HLD133		HLD133-I			
HLD79		HLD79-I			
HLD105	HLD105-D	HLD105-I			
HLD140		HLD140-I			
HLD163	HLD163-D	HLD163-I			
BTG Panel					
HLD91	HLD91-D	HLD91-I			
HLD23		HLD23-I			
HLD88	HLD88-D	HLD88-I			
HLD101	HLD101-D	HLD101-I			
HLD67	HLD67-D	HLD67-I			
HLD301	HLD301-D	HLD301-I			
HLD53	HLD53-D	HLD53-I			
HLD97		HLD97-I			
HLD152	HLD152-D				
HLD128	HLD128-D	HLD128-I			
HLD134	HLD134-D	HLD134-I			
HLD305	HLD305-D	HLD305-I			

Kit content

Mentype® **DIPquant** (100 reactions)

Nuclease-free water	3.0 ml
Reaction mix **D**	500 µl
Primer mix	250 µl
Multi Taq2 DNA polymerase	40 µl

Ordering information

Mentype® **DIPquant**	25 reactions	Cat. No.	See Table 3
Mentype® **DIPquant**	50 reactions	Cat. No.	See Table 3
Mentype® **DIPquant**	100 reactions	Cat. No.	See Table 3

Storage

Store all components at -20 °C and avoid excessive freeze-thaw cycles. Primer mix and ROX Passive Reference must be stored protected from light. The DNA samples should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents available for Biotype® qPCR assay analysis:

Reagent	Supplier	Order number
General Positive Control	Biotype Diagnostic GmbH	00-10020-0100
ROX Passive Reference	Biotype Diagnostic GmbH	00-30720-0200

Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Reaction mix D	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request.
Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic 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.

Trademarks and patents

Mentype® is a registered trademark of Biotype Diagnostic GmbH.

2. Outline of working steps performed with Mentype® DIP-products

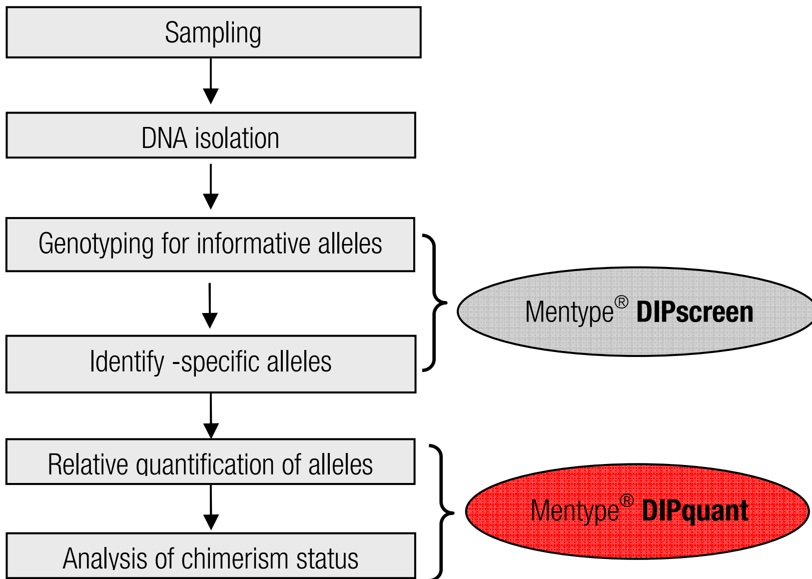

Fig. 1 From sample to analysis – Monitoring chimerism with the Mentype® **DIPscreen** and the Mentype® **DIPquant** assay

Protocols for qPCR amplification and analysis

3. qPCR amplification

3.1 Master mix preparation

The table below shows volumes of applied reagents per 5.0 µl sample volume (template-DNA) in a total reaction volume of 25 µl. Please adjust the volume of water according to the amount of template DNA applied. The number of reactions to be set up shall be determined taking into account the number of replicates as well as positive and negative control reactions. Add one or two reactions to this number to compensate pipetting errors.

Component	Volume
Nuclease-free water	12.1 µl
Reaction mix **D***	5.0 µl
Primer mix	2.5 µl
Multi Taq2 DNA Polymerase (hot start, 2.5 U/µL)	0.4 µl
Volume of master mix	20.0 µl

* contains Mg^{2+}, dNTPs, BSA, if required add ROX Passive Reference before use

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The volume of DNA applied to the assays depends on its concentration. For reference samples 5 µl is mostly sufficient. For critical patients samples the amount of template-DNA might be increased appropriately. Adjust the final reaction volume to 25 µl with nuclease-free water.

Generally, DNA-templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCL, pH 8.0 and 1 mM EDTA), e.g. 0.1 x TE buffer.

Detection limit and sensitivity of the Mentype® **DIPquant** analysis is mostly depending on quality and quantity of applied DNA. The allele-specific Primer mix is optimized for high specificity and sensitivity if 250 ng of purified total DNA is applied. This refers to a total cell equivalent of 41.666 cells (6 pg/cell). No unspecific background should appear < 43 cycles in donor DNA with -specific assays. The detection limit of the Mentype® **DIPquant** assays is about 31 pg (5 cells) for homozygote DNA and 65 pg (10 cells) for heterozygote DNA.
An input of higher amounts of DNA (> 250 ng up to 500 ng) might increase sensitivity. However, sporadic amplification of unspecific signals < 43 cycles in donor DNA may occur. We recommend a pre-transplant test of the donor DNA to ensure specificity in such cases.

Detection sensitivity and required input of DNA amounts

Input DNA amount (ng)	Cell equivalents (6pg/cell)	Sensitivity limit (%) for homozygote allele constellation (type 1)	Sensitivity limit (%) for heterozygote allele constellation (type 2)
500	83333	0.006	0.012
250	41666	0.012	0.024
100	16666	0.03	0.06
50	8333	0.06	0.12
25	4166	0.12	0.24
10	1666	0.3	0.6
5	833	0.6	1.2

ROX Passive Reference (separate component)

Some real-time thermocyclers need the ROX Passive Reference to normalize well-to-well variations resulting from pipetting errors or instrument limitations. Please add ROX Passive Reference in an appropriate volume to Reaction Mix **D** (500µl) before preparing the master mix. When stored at -20 °C the mixture is stable for at least 6 month.

Real-time thermocycler	Volume ROX within REM D
MX3000P® / MX3005P®/ MX4000®	11 µl to 500 µl REM **D**
ABI Prism® 7500 / FAST 7500	3.85 µl to 500 µl REM **D**
ABI Prism® 5700/ 7000/ 7300/ 7700/ 7900/ FAST 7900	
ABI Step One/One Step Plus	55 µl to 500 µl REM **D**
Mastercycler® ep realplex I/II (Firmware 2.1 or below)	

Positive control (separate component)

For the positive amplification control, apply 5 µl of General Positive Control (1 ng/µl) instead of template DNA and add it to reaction-tubes containing the qPCR master mix. Because the copy number of specific alleles differs within the control, resulting Cp/Ct values are expected between 27 and 31 depending on the analysed DIP assay (see Table 1).

Negative control

For the negative amplification control, pipette 5 µl nuclease-free water instead of template DNA into the reaction tube containing the qPCR master mix.

Template DNA

Detection limit and sensitivity of the Mentype® **DIPquant** analysis is mostly depending on quality and quantity of applied DNA. For DNA isolated from peripheral blood we recommend to apply 250 ng of total DNA to achieve optimal results. For DNA isolated from sorted cell populations choose adequate DNA amounts to analyse samples (see 3.1). Sometimes, measured DNA concentration varies depending on the quantification method used. In this instant it might be necessary to adjust the optimal DNA amount.

3.2 qPCR setup

To set up the qPCR, transfer 20 µl of master mix into each well of optical reaction tubes or optical multi-well reaction plates.
It is recommended to use white PCR plates or tubes for qPCR analysis if available for the respective real-time thermocycler. The white color virtually eliminates cross talk and improves the efficiency of fluorescent detection thereby increasing assay sensitivity and well-to-well consistency.

Recommended plate setup

For relative quantification of chimerism status we recommend four different qPCR assays: one for the **active reference (REF)** and **three** for different **-specific alleles** (**allele of interest**, **AOI**). We recommend at least **two replicates** per DNA for reference and the -specific assays, respectively. For each assay a **negative (NTC)** and **positive control (PC)** should be performed.

If the total amount of DNA, or, the number of -specific alleles is limited analysis of only two -specific alleles (allele of interest, AOI) is likewise possible.

Quantification before transplantation (pre-HSCT)

To calibrate the analysis, DNA that was isolated before transplantation (Calibrator) should be analysed with the reference gene and the preselected -specific assay. This quantification is set as the 100 % level.
To ensure specificity for the allele-specific assay, donor DNA may be analysed additionally and may set as the 0 % level.

Setup example for 96-well formats before transplantation (Calibrator)

	REF	AOI-1	AOI-2	AOI-3	
	Rep. 1	Rep. 1	Rep. 1	Rep. 1	
	Rep. 2	Rep. 2	Rep. 2	Rep. 2	
	NTC REF	NTC AOI-1	NTC AOI-2	NTC AOI-3	
	PC REF	PC AOI-1	PC AOI-2	PC AOI-3	
		AOI-1 Donor	***AOI-2 Donor***	***AOI-3 Donor***	
		Rep. 1	*Rep. 1*	*Rep. 1*	
		Rep. 2	*Rep. 2*	*Rep. 2*	
		optional controls for donor			

Quantification after transplantation / monitoring (post-HSCT)

Follow-up analysis is performed with patient DNA freshly isolated at respective time points after transplantation. Again, reference gene as well as -specific assays shall be analysed. Add a negative and positive control to each quantification reaction.

Setup example for 96 well formats after transplantation (Monitoring)*

	REF	AOI-1	AOI-2	AOI-3	
	Rep. 1	Rep. 1	Rep. 1	Rep. 1	
	Rep. 2	Rep. 2	Rep. 2	Rep. 2	
	NTC REF	NTC AOI-1	NTC AOI-2	NTC AOI-3	
	PC REF	PC AOI-1	PC AOI-2	PC AOI-3	

* in case of analysing retrospective samples referring to different monitoring time points on the same plate the number of NTCs, PCs might be reduced.

After pipetting, seal the tubes or the plate with optical caps or optical sealing foil. Centrifugate briefly and load samples to the instrument.

3.3 Instrument set up and amplification parameter

Create an experiment for the run using the qPCR detection and amplification parameters stated below.
For detailed instructions please refer to the user manual and technical documentation of the respective instrument.

Detection parameter

6-FAM serves as reporter dye for all assays. Please select the respective filterset in the real-time instrument software.

If required choose ROX as passive reference dye for normalization on your instrument (see 3.1).

qPCR amplification parameter*

In order to prevent the formation of non-specific amplification products "hot start" Multi Taq2 DNA Polymerase is used.

Temperature	Time	
94°C	4 min (hot start to activate Multi Taq2 DNA Polymerase)	
94°C	30 sec	45 cycles
62°C	**45 sec**	

* Validated on Roche Light Cycler LC480 (standard ramping rates of about 4.4 °C/sec for heating and 2.2 °C/sec for cooling) and ABI 7000 (9600 ramping was deactivated).

Data-collection should be performed at annealing and elongation step at 62 °C.

Write a sample list according to your chosen experimental setup. If applying Chimeris™**Monitor** for subsequent quantification procedure we recommend including the DIPquant assay name within the sample name (e.g. Patient 300_Day28_HLD104-D_Rep.1). This mediates automatic assay recognition in Chimeris™**Monitor**.

4. Analysis

Data analysis

View the amplification plots for the entire plate. Detailed analysis of raw data depends on the real-time PCR instrument used. Baseline noise levels should either be set automatically or at predefined cycles (e.g. 3-15). Please use the NTC to set an appropriate threshold. As ΔΔCp method is performed for quantification, individual threshold settings have no influence on the final results.

Please refer to the instrument-specific user guide regarding instructions of how to analyse data and of how to export results as tab. delimited txt. file for further usage with Chimeris™**Monitor** or Excel. Please export at least the following columns (Sample name, Cp/Ct value)

Verification of results

The run is valid if the Cp/Ct of positive control is below < 33 cycles (see Tab. 2) and the negative control shows no amplification < 45 cycles.
When using donor DNA to control specificity of the allele-specific assay no signals < 43 cycles should be detectable.

5. Quantification

After analysis of amplification plots and exporting corresponding Cp/Ct values, quantification of informative DIP-alleles and monitoring can be performed with Chimeris™**Monitor** Software from Biotype Diagnostic GmbH. Please refer the Chimeris™**Monitor** user guide for further instructions.

Manual quantification of qPCR data should be performed with the relative quantification method. As ΔΔCp/Ct method is used for quantification, individual threshold settings have no influence on final results. Please use the NTC to set an appropriate threshold.

Calculation

Quantification of Pre-HSCT samples (Cp corresponds to Ct)

1. Calculate individual Cp values of DNA for reference (REF) and allele of interest (AOI).
2. Calculate ΔCp for each AOI to REF gene (ΔCp C = Cp AOI – Cp REF)
3. This ΔCp is used as calibrator value (ΔCp C) in post-HSCT calculations (100%)

Quantification of Post-HSCT samples (Cp corresponds to Ct)

1. Calculate individual Cp values of DNA for reference (REF) and allele of interest (AOI)
2. Calculate ΔCp for each AOI to REF gene (ΔCp U = Cp AOI – Cp REF)
3. This ΔCp is used further in calculation for the unknown status (ΔCp U)
4. Calculate ΔΔCp for quantification of chimerism (ΔΔCp = ΔCp U – ΔCp C)
5. Calculate % of component depending on qPCR efficiency % recipient = $((1+E)^{-(\Delta\Delta Cp)}) \times 100$. In case of qPCR efficiency of 100 % use reduced formula $(2^{-(\Delta\Delta Cp)}) \times 100$.

Table 2: Expected Cp/Ct values for positive control amplification and calculated assay efficiencies

DIPquant assay	Expected Cp/Ct with 5 ng GPC*	qPCR Efficiency (E)**
Reference	27	0.95
SRY	29	1.00
SMCY	29	1.00
HLD23-I	27.5	1.00
HLD38-I	28	1.00
HLD48-I	28.5	1.00
HLD53-D	30	1.00
HLD53-I	29	1.00
HLD67-D	28	0.98
HLD67-I	30	0.98
HLD70-D	28	1.00
HLD70-I	28	0.97
HLD79-I	28.5	1.00
HLD82-D	28	1.00
HLD82-I	27.5	1.00
HLD84-D	30.5	0.93
HLD84-I	29	0.95
HLD88-D	29	1.00
HLD88-I	27.5	1.00
HLD91-D	28	0.92
HLD91-I	27	0.99
HLD97-I	28	1.00
HLD101-D	28	0.97
HLD101-I	30	1.00
HLD103-D	29	0.99
HLD103-I	28.5	0.91
HLD104-D	27.5	1.00
HLD104-I	29	1.00
HLD105-D	27.5	1.00
HLD105-I	28	0.95

DIPquant assay	Expected Cp/Ct with 5 ng GPC*	qPCR Efficiency (E)**
HLD106-D	29.5	1.00
HLD106-I	30	0.92
HLD110-I	28.5	0.97
HLD112-I	28	1.00
HLD114-D	28	0.90
HLD114-I	29	1.00
HLD116-D	28	0.93
HLD116-I	27	1.00
HLD128-D	29	0.92
HLD128-I	28	1.00
HLD131-D	27.5	0.95
HLD131-I	29	0.90
HLD133-I	28	1.00
HLD134-D	27	0.95
HLD134-I	27.5	0.92
HLD140-I	30	1.00
HLD152-D	29.5	1.00
HLD163-D	27	1.00
HLD163-I	28.5	1.00
HLD301-D	30	1.00
HLD301-I	28.5	1.00
HLD304-D	30	1.00
HLD305-D	27.5	1.00
HLD305-I	29	0.98
HLD307-D	27.5	1.00
HLD307-I	29.5	0.92
HLD310-D	28	0.90
HLD310-I	28	0.90

* Absolute quantification run on Roche Light Cycler LC480, analysed with 2nd Derivative Maximum Method
** Experimentally determined by serial dilution series of homozygote DNA, calculated values > 1.00 were set to 1.00.

6. Interpretation of results

Poor signal or no signal detected

One or more reaction components were not added
Verify your master mix and sample setup. Check positive control performance.
Rerun the assay if necessary.

Wrong allele-specific assay was used
Ensure that the right Mentype® **DIPquant** assays was applied for right -specific allele.

Suboptimal thermocycling conditions
Verify conditions for the qPCR amplification. Ensure the initial 94 ºC activation step was carried out correctly for full Multi Taq2 DNA polymerase activation. Verify annealing and extension temperature. Please check heating and cooling rates of your thermocycler and adjust to 4-4 °C/sec for heating and 2.2 °C/sec for cooling.

Inhibitors are present
Sometimes inhibitors are carried over from nucleic acid extraction. Take care during the nucleic acid extraction steps to minimize carryover of PCR inhibitors. You may purify or dilute the template DNA in order to rule out presence of PCR inhibitors. Rerun the assay with the purified template.

Data collection failure
Ensure that fluorescent data is collected at the appropriate step and in the correct channel. Check that machine settings correspond with the dye conjugated to the fluorescent probe and that ROX / fluorescein levels are correct.

Baseline or threshold problems

Set threshold above noise level to obtain accurate Cp/Ct values.

Refer to the appropriate user guide of your real-time instrument for correct procedure. Try to set the baseline and threshold manually instead of automatic.

Degradation of template DNA
Degradation can occur during sample preparation or storage. Store diluted template in 1x or 0.1x TE buffer. Use the Positive Control to ensure overall assay performance.

Degradation of qPCR components
Check the expiry date of all components. Verify the handling and storage conditions. Please avoid excessive freeze-thaw cycles of the Primer mix and store all components at –20 °C.

Poor reproducibility across replicate samples

Pipetting errors
Ensure that no pipetting errors occur during the process. You may prevent variation due to pipetting error by wearing gloves, using aerosol-resistant filter tips and calibrated pipettes.

Variations in master mix and template addition
Always prepare a master mix with sufficient volume to prepare all replicate samples. Ensure that reaction components were mixed thoroughly. Upon pipetting components for the master mix and reaction components should be mixed by briefly vortexing and a subsequent centrifugation (10 s). Please avoid pipetting less than 5 µl of template.

Inhibitors are present
Sometimes inhibitors are carried over from nucleic acid extraction. The presence of inhibitors is very likely to cause suboptimal reactions with poor reproducibility.
You may re-purify or dilute the template DNA in order to rule out presence of PCR inhibitors. Take care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.

Baseline or Threshold was set improperly
Set threshold above noise level to obtain accurate Cp/Ct values.
Refer to the appropriate user guide of your real-time instrument for correct procedure. Try to set baseline or threshold manually instead of automatic.

Too low or high ROX level
Some real-time thermocyclers need the ROX Passive Reference to normalize well-to-well variations resulting from pipetting errors or instrument limitations.
Please ensure that right amounts were added to the Reaction mix D before setting up the master mix.

Too low concentration of target-DNA - limit of sensitivity
Sensitivity of the assays was determined to at least 5-10 cell equivalents. Lower levels of DNA may reduce reproducibility between replicates.

Signals within negative control

Please run a no-template control to verify the absence of contamination.
In order to rule out cross-contaminations use barrier tips, screw-cap tubes and setup qPCR reaction in a DNA-free zone before adding the template DNA in a separate location.

Signals of specific assays in donor DNA sample

Too much template DNA applied

Unspecific amplification of specific alleles within donor DNA might occur if >250ng of total DNA were applied. In this case reduce the DNA input to 250 ng and re-run the assay. Please note that the input should not exceed 500 ng of template DNA.

False negative genotyping of donor due to mutational events

Allelic dropouts may occur due to DNA specific mutations appearing at the primer binding site deployed for the Mentype® **DIPscreen** multiplex and might result in false negative allele detection. As primer binding sites for Mentype® **DIPquant** assays differ positive signal might be observed in qPCR.

Amplification curve show low or no ROX dye (passive reference)

Some real-time thermocyclers need the ROX Passive Reference to normalize well-to-well variations resulting from pipetting errors or instrument limitations. Please ensure that right amounts of ROX were added to Reaction mix **D** before setting up the master mix. Please ensure ROX was chosen as passive reference dye in the instrument software.

7. References

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Table 3: Ordering information for allele-specific DIPquant assays

DIPquant Assay	25 Reactions	50 Reactions	100 Reactions
Reference	45-01591-0025	45-01591-0050	45-01591-0100
SRY	45-01590-0025	45-01590-0050	45-01590-0100
SMCY	45-01589-0025	45-01589-0050	45-01589-0100
HLD23-I	45-01538-0025	45-01538-0050	45-01538-0100
HLD38-I	45-01558-0025	45-01558-0050	45-01558-0100
HLD48-I	45-01560-0025	45-01560-0050	45-01560-0100
HLD53-D	45-01561-0025	45-01561-0050	45-01561-0100
HLD53-I	45-01562-0025	45-01562-0050	45-01562-0100
HLD67-D	45-01567-0025	45-01567-0050	45-01567-0100
HLD67-I	45-01568-0025	45-01568-0050	45-01568-0100
HLD70-D	45-01569-0025	45-01569-0050	45-01569-0100
HLD70-I	45-01570-0025	45-01570-0050	45-01570-0100
HLD79-I	45-01576-0025	45-01576-0050	45-01576-0100
HLD82-D	45-01577-0025	45-01577-0050	45-01577-0100
HLD82-I	45-01578-0025	45-01578-0050	45-01578-0100
HLD84-D	45-01579-0025	45-01579-0050	45-01579-0100
HLD84-I	45-01580-0025	45-01580-0050	45-01580-0100
HLD88-D	45-01581-0025	45-01581-0050	45-01581-0100
HLD88-I	45-01582-0025	45-01582-0050	45-01582-0100
HLD91-D	45-01585-0025	45-01585-0050	45-01585-0100
HLD91-I	45-01586-0025	45-01586-0050	45-01586-0100
HLD97-I	45-01588-0025	45-01588-0050	45-01588-0100
HLD101-D	45-01501-0025	45-01501-0050	45-01501-0100
HLD101-I	45-01502-0025	45-01502-0050	45-01502-0100
HLD103-D	45-01505-0025	45-01505-0050	45-01505-0100
HLD103-I	45-01506-0025	45-01506-0050	45-01506-0100
HLD104-D	45-01507-0025	45-01507-0050	45-01507-0100
HLD104-I	45-01508-0025	45-01508-0050	45-01508-0100
HLD105-D	45-01509-0025	45-01509-0050	45-01509-0100
HLD105-I	45-01510-0025	45-01510-0050	45-01510-0100
HLD106-D	45-01511-0025	45-01511-0050	45-01511-0100
HLD106-I	45-01512-0025	45-01512-0050	45-01512-0100
HLD110-I	45-01514-0025	45-01514-0050	45-01514-0100
HLD112-I	45-01516-0025	45-01516-0050	45-01516-0100
HLD114-D	45-01517-0025	45-01517-0050	45-01517-0100

HLD114-I	45-01518-0025	45-01518-0050	45-01518-0100
HLD116-D	45-01519-0025	45-01519-0050	45-01519-0100
HLD116-I	45-01520-0025	45-01520-0050	45-01520-0100
HLD128-D	45-01523-0025	45-01523-0050	45-01523-0100
HLD128-I	45-01524-0025	45-01524-0050	45-01524-0100
HLD131-D	45-01525-0025	45-01525-0050	45-01525-0100
HLD131-I	45-01526-0025	45-01526-0050	45-01526-0100
HLD133-I	45-01528-0025	45-01528-0050	45-01528-0100
HLD134-D	45-01529-0025	45-01529-0050	45-01529-0100
HLD134-I	45-01530-0025	45-01530-0050	45-01530-0100
HLD140-I	45-01532-0025	45-01532-0050	45-01532-0100
HLD152-D	45-01533-0025	45-01533-0050	45-01533-0100
HLD163-D	45-01535-0025	45-01535-0050	45-01535-0100
HLD163-I	45-01536-0025	45-01536-0050	45-01536-0100
HLD301-D	45-01539-0025	45-01539-0050	45-01539-0100
HLD301-I	45-01540-0025	45-01540-0050	45-01540-0100
HLD304-D	45-01541-0025	45-01541-0050	45-01541-0100
HLD305-D	45-01543-0025	45-01543-0050	45-01543-0100
HLD305-I	45-01544-0025	45-01544-0050	45-01544-0100
HLD307-D	45-01545-0025	45-01545-0050	45-01545-0100
HLD307-I	45-01546-0025	45-01546-0050	45-01546-0100
HLD310-D	45-01549-0025	45-01549-0050	45-01549-0100
HLD310-I	45-01550-0025	45-01550-0050	45-01550-0100

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