ExaccType DPYD Kit

Handbook

RUO For research use only. Not for use in diagnostic procedures.

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Notice of Change

Please note the following adaptions compared to the previous handbook version:

Document code	Changes	Date
DPYDHB01v1en	**Initial version**	**22.10.2026**

For any further questions, please contact us

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Intended Use

ExaccType DPYD Kit is intended to determine *DPYD* genotype from extracted genomic deoxyribonucleic acid (gDNA), or directly from human-derived EDTA whole blood samples and is to be used in basic, pharmaceutical, or chemical research. **The ExaccType DPYD Kit is not to be used in diagnostic procedures.**

ExaccType DPYD Kit is designed to detect the following *DPYD* alleles (Table 1).

Table 1: Alleles detected by the ExaccType DPYD Kit.

Variant (NM_000110.4)	rsID
c.1905+1G>A	rs3918290
c.1679T>G	rs55886062
c.1129-5923C>G	rs75017182
c.557A>G	rs115232898
c.2846A>T	rs67376798
c.868A>G	rs146356975
c.2279C>T	rs112766203

Product description

The ExaccType DPYD Kit enables the qualitative detection of specific *DPYD* gene variants using a high throughput, multiplexed real-time PCR format. The kit supports two sample types: extracted genomic DNA (gDNA) and EDTA whole blood. When using EDTA whole blood, the sample should be diluted to 1% (v/v) in nuclease-free water prior to testing. A total of 20 µL of prepared sample is required to assess all variants detected by the kit.

The kit is delivered in a multiplex format, comprising four Primer Probe mixes (PP mixes). Each PP mix includes:

- Up to four allele-specific probes for variant and wildtype detection
- Up to two PCR primer pairs designed to amplify regions of interest with high specificity

These components have been carefully optimised to distinguish between wildtype and variant alleles with high analytical specificity. Each PP mix targets a defined set of *DPYD* alleles, and fluorophore labelling allows for clear differentiation of each allele type during real-time PCR analysis.

Details of the *DPYD* variants detected and the corresponding fluorophores used in each PP mix are provided in Table 2.

Table 2: Variants/Alleles detected by each assay.

PP Mix	Variant (NM_000110.4)	Nucleic acid base detected	Alleles detected	Fluorophore
1	c.1905+1G>A	G	Wildtype	HEX™
		A	Variant	FAM™
	c.1679T>G	T	Wildtype	ATTO™550
		G	Variant	ROX
2	c.1129-5923C>G	C	Wildtype	HEX™
		G	Variant	FAM™
3	c.557A>G	A	Wildtype	HEX™
		G	Variant	FAM™
	c.2846A>T	A	Wildtype	ATTO™550
		T	Variant	ROX
4	c.868A>G	A	Wildtype	HEX™
		G	Variant	FAM™
	c.2279C>T	C	Wildtype	ATTO™550
		T	Variant	ROX

The probes are labelled with a 5' reporter dye (FAM™, HEX™, ROX, or ATTO™550) and a downstream 3' dye-free quencher. When a probe is intact the quencher results in suppression of the reporter fluorescence primarily by Förster-type energy transfer. The probes hybridise to the target sequence between the primers and during qPCR, the DNA polymerase enzyme cleaves the probe between the reporter and the quencher leading to an increase in detectable reporter fluorescence. This process occurs in every qPCR cycle. The increase in fluorescence is directly proportional to the target amplification during qPCR.

Materials provided

Kit Contents

ExaccType DPYD Kit
(36 samples in singlicate + controls)

Component	Colour	Volume
Primer Probe Mix 1	Red	1x 250µL
Primer Probe Mix 2	Orange	1x 250µL
Primer Probe Mix 3	Yellow	1x 250µL
Primer Probe Mix 4	Green	1x 250µL
Enzyme Mix	White	2x 1000µL
Positive Control (PC)	Black	1x 210µL
No Template Control (NTC)	Clear	1x 210µL

Material and devices required but not provided

Consumables

- Sterile pipette tips with filters
- Sterile microcentrifuge tubes
- PCR plates/seals or tubes compatible with a qPCR instrument

General laboratory equipment

- Real-Time PCR instrument (calibrated for FAM™, HEX™, ROX, and ATTO™550 fluorophores). Refer to the equipment user guide for further information on instrument calibration.
- Calibrated adjustable volume pipettes
- Centrifuge (for microcentrifuge tubes)
- Plate centrifuge
- Vortex
- Adhesive Film Applicator

Reagents

- Nuclease free water for EDTA whole blood dilution

Reagent storage and handling

- If the kit is not frozen on arrival, the outer packaging is damaged or if any component of the kit is not present, please contact BIOTYPE GmbH
- Store kit immediately on receipt at –25 °C to –15 °C in a constant-temperature freezer and protected from light
- Reagent stability studies are currently ongoing the initial kit stability claim will be included in a future version of this handbook
- Repeated thawing and freezing should be avoided
- To ensure optimal activity and performance, PP mixes must be protected from light to avoid photo bleaching
- Do not use expired or incorrectly stored components

Warnings and precautions

This product is for **research use only**. Not intended for any diagnostic, clinical or medical purpose or objective.

General Precautions

- Avoid multiple freeze thaws of sample prior to testing with the ExaccType DPYD Kit
- All PP Mixes should be tested with each sample to minimise the possibility of obtaining false negative results
- Discard any samples or waste according to local safety procedures
- Reagents in the ExaccType DPYD Kit have been diluted optimally. Do not dilute reagents further
- Controls provided should be tested with all reaction mixes on each qPCR run and are necessary to confirm correct ExaccType DPYD Kit performance and absence of contamination
- Take extreme care to prevent contamination of the kit components with the Positive Control reagent. Cap the tubes promptly after addition of each reagent
- Take extreme care to prevent cross-contamination between samples. Cap the tubes promptly after addition of each sample and change tips between different samples

- Care should be taken to maintain sample traceability; sample tubes should be clearly labelled, and care should be taken when inputting sample information into the run template
- Do not remove the plate seal after the run has finished

Safety Information

- When working with chemicals, always wear suitable personal protective equipment (lab coat, disposable gloves, and protective eyewear). For more information, please consult the appropriate Non-Hazardous Product Statement (NHS) or safety data sheets (SDS).
- EDTA whole blood should be treated as potentially biohazardous and set-up of the qPCR with this sample type should be performed in a Class II Microbiological Safety cabinet.
- Any biohazardous waste should be disposed of according to local safety procedures.

Procedure

Sample preparation

Whole Blood Preparation

Whole blood (EDTA) should be diluted to 1% (v/v) using nuclease free water prior to testing with the ExaccType DPYD Kit.

Before performing the dilution homogenise the whole blood sample by gently inverting the collection tube several times. To ensure accurate pipetting and to avoid pipetting small volumes, prepare a minimum of 200 µL of diluted blood per sample e.g. 2 µL whole blood in 198 µL nuclease free water. Vortex the diluted sample and briefly spin to collect.

During aspiration and dispensing, use very slow plunger movements and hold the tips in the liquid for a few seconds at the end of both aspiration and dispensing. Dilution of blood samples should be performed immediately prior to plate set up. Diluted material should not be stored, if a sample requires to be re-run a new dilution of blood should be performed.

DNA Sample Preparation

DNA should be extracted from samples using an extraction kit suitable for the purification of gDNA. Ensure that the chosen extraction kit is used according to the manufacturer's instructions. The input range of the assay is between 0.30-150 ng total gDNA input per reaction. 20 µL of gDNA at a concentration between 0.06 – 30 ng/µL is sufficient to assess all variants detected by the kit. Quantification of gDNA samples can be performed by using an appropriate quantification method (e.g. Fluorometer or UV spectrometer).

Plate Set Up & Cycling

The master mix contains all the components required for qPCR, except the template DNA or diluted whole blood. Controls provided with the kit should be included in every run (negative control and positive control).

The kit is sufficient for testing 36 samples across up to four PCR plates. A maximum of 22 samples can be analysed in a single qPCR run. An example set-up is shown in Figure 1. The following batch sizes are recommended:

- 4 PCR plates with 9 samples each
- 3 PCR plates with 12 samples each
- 2 PCR plates with 18 samples each

Thaw all kit components and samples (if frozen). It is important to mix the solutions completely before use to avoid localised differences in concentration, briefly vortex and spin to collect.

Reagent preparation and PCR set up can be performed at room temperature.

Master mix setup

Prepare enough volume of master mix for one technical replicate per sample, one PC, one NTC, and one additional replicate (n+1) per mix to allow sufficient overage volume for PCR setup.

For each of the PP Mixes, prepare master mixes in microcentrifuge tubes immediately before use as per Table 3, adjusting the volumes depending on the required number of reactions. Using a vortex/centrifuge, mix the master mixes for at least 5 seconds and centrifuge to collect the volume at the bottom of the tube.

Table 3: Master mix manufacture for N=1 sample.

Master Mix ID	Volume of Enzyme Mix per reaction (µL)	Primer Probe Mix	Volume of Primer Probe Mix per reaction (µL)
Mix 1	10	**PP mix 1**	5
Mix 2	10	**PP mix 2**	5
Mix 3	10	**PP mix 3**	5
Mix 4	10	**PP mix 4**	5

Reaction Set Up

- Samples should be mixed for 5 seconds and then centrifuged briefly to collect
- Pipette 15 µL of the corresponding Master Mix into each well changing tips between each mix
- Pipette 5 µL of Sample/PC or NTC into each well changing a tip between each addition
- To reduce a risk of cross contamination, negative and positive controls should always be positioned on the right side of the plate
- The final concentration of components per reaction is detailed in Table 4

Table 4: Final concentration of components per reaction.

Component	Volume/reaction (µL)	Final concentration
Enzyme Mix	10.0	1x
Primer Probe Mix	5.0	1x
Master Mix Total	15.0	-
Sample (1% diluted Blood/gDNA/PC/NTC)	5.0	Variable
Total Reaction Volume	20.0	-

- Seal the plate using a PCR plate seal and sealing tool
- Mix the plate/tubes by vortexing for one minute
- Centrifuge the plate/tubes for >1 minute to spin contents to bottom of the plate/tubes
- Visually assess for bubbles, if any are present, flick the plate/tubes and centrifuge for additional 30 seconds. Repeat until no bubbles are present
- Place the plate/tubes into the compatible real time PCR instrument following the manufacturer's instructions

	1	2	3	4	5	6	7	8	9	10	11	12
A	Mix 1 Sample 1	Mix 1 Sample 2	Mix 1 Sample 3	Mix 1 Sample 4	Mix 1 Sample 5	Mix 1 Sample 6	Mix 1 Sample 7	Mix 1 Sample 8	Mix 1 Sample 9		Mix 1 PC	Mix 1 NTC
B	Mix 2 Sample 1	Mix 2 Sample 2	Mix 2 Sample 3	Mix 2 Sample 4	Mix 2 Sample 5	Mix 2 Sample 6	Mix 2 Sample 7	Mix 2 Sample 8	Mix 2 Sample 9		Mix 2 PC	Mix 2 NTC
C	Mix 3 Sample 1	Mix 3 Sample 2	Mix 3 Sample 3	Mix 3 Sample 4	Mix 3 Sample 5	Mix 3 Sample 6	Mix 3 Sample 7	Mix 3 Sample 8	Mix 3 Sample 9		Mix 3 PC	Mix 3 NTC
D	Mix 4 Sample 1	Mix 4 Sample 2	Mix 4 Sample 3	Mix 4 Sample 4	Mix 4 Sample 5	Mix 4 Sample 6	Mix 4 Sample 7	Mix 4 Sample 8	Mix 4 Sample 9		Mix 4 PC	Mix 4 NTC
E												
F												
G												
H												

Figure 1: Suggested Mix and Sample Layouts.

Run Method

NOTE

Prior to set up ensure the instrument is calibrated for the dyes required for this experiment.

NOTE

When using an instrument other than the QuantStudio™5, instrument-specific settings and data analysis parameters may differ. For guidance on the appropriate setup and analysis settings for your platform, please contact support@biotype.de.

Refer to the selected real-time PCR platform user manual to set up the PCR run. The recommended qPCR cycling parameters are outlined in Table 5.

NOTE

A passive reference dye is not included in the kit — passive reference dye normalisation **should not** be selected.

Table 5: Recommended cycling parameters for the ExaccType DPYD Kit.

Step	Step Number	Step Name	Temp	Time	Ramp Rate	Cycles
Hold Stage	1	Initial Activation	95°C	10 min	1.6°C/s	1
PCR Stage	1	Denaturation	94°C	10 s	1.6°C/s	40
	2	Annealing Extension Data Acquisition	60°C	30 s	1.6°C/s	

Threshold Setting

Targets, reporter dyes and quenchers for each PP Mix are shown in Table 6. Automatic baseline and threshold settings should be used. Users are recommended to empirically determine the suitability of thresholds for the qPCR instrument under use. The threshold should be set at the level of detection, or the point at which a reaction reaches a fluorescent intensity above background levels. For use with the Applied Biosystems™ QuantStudio™5 Dx (QS5 Dx) instrument we recommend thresholds provided in Table 6.

For further information of threshold setting on different qPCR platforms refer to chapter Appendix A: Threshold Setting.

Table 6: Dye, Quencher and Threshold information for targets included in the ExaccType DPYD Kit.

PP Mix	Variant (NM_000110.4)	Nucleic acid base detected	Alleles detected	Fluorophore	Quencher*	ΔRn Threshold**
1	c.1905+1G>A	G	Wildtype	HEX™	EDQ-MGB	15,000
		A	Variant	FAM™	EDQ-MGB	35,000
	c.1679T>G	T	Wildtype	ATTO™550	EDQ-MGB	10,000
		G	Variant	ROX	EDQ-MGB	15,000
2	c.1129-5923C>G	C	Wildtype	HEX™	Iowa Black FQ	25,000
		G	Variant	FAM™	Iowa Black FQ	35,000
3	c.557A>G	A	Wildtype	HEX™	EDQ-MGB	15,000
		G	Variant	FAM™	EDQ-MGB	40,000
	c.2846A>T	A	Wildtype	ATTO™550	EDQ-MGB	20,000
		T	Variant	ROX	EDQ-MGB	20,000
4	c.868A>G	A	Wildtype	HEX™	EDQ-MGB	15,000
		G	Variant	FAM™	EDQ-MGB	40,000
	c.2279C>T	C	Wildtype	ATTO™550	EDQ-MGB	15,000
		T	Variant	ROX	EDQ-MGB	15,000

* Quencher is provided for information only; not required for run settings

** Thresholds provided for use with QS5 Dx instrument

Run Export

Once the run is completed export the Ct (depending on instrument these may also be referred to as Cq or Cp) values. Refer to the selected real-time PCR platform user manual for analysis and export instructions.

Analysis can be completed either manually, as outlined below, or with the provided analysis spreadsheet. The spreadsheet includes recommended run and sample settings and is best suited for data from the QS5 Dx instrument.

Recommended Run Validity Criteria

NOTE

Ensure Ct values come from true amplification (sigmoidal amplification curves) rather than artifacts (linear or irregular curves) by examining the normalised fluorescence plot. Adjust the threshold and baseline settings as necessary, following the instructions in the qPCR instrument manual. Treat any data from artifacts as no amplification (see chapter Results Interpretation).

NOTE

If using the QS5 Dx instrument the Amp status can be used to determine true amplification.

The run is deemed valid when the results for the negative control for all targets produce no amplification and each target amplifies within the positive control. The run validity criteria for use with the QS5 Dx instrument are outlined in Table 7. PC validity criteria should be verified if using an alternate instrument.

Table 7: Recommended Run Validity Criteria for the ExaccType DPYD Kit.

Reaction Mix	Variant (NM_000110.4)	Nucleic acid base detected	Alleles detected	Fluorophore	Run Validity Criteria	
					Acceptable Positive Control (PC) Ct range	Negative Control (NTC)
Mix 1	c.1905+1G>A	G	Wildtype	HEX™	≥ 24.73 – ≤ 33.73	Undetermined
		A	Variant	FAM™	≥ 24.71 – ≤ 33.71	
	c.1679T>G	T	Wildtype	ATTO™550	≥ 23.24 – ≤ 32.24	
		G	Variant	ROX	≥ 25.30 – ≤ 34.30	
Mix 2	c.1129-5923C>G	C	Wildtype	HEX™	≥ 24.38 – ≤ 33.88	
		G	Variant	FAM™	≥ 23.96 – ≤ 33.46	
Mix 3	c.557A>G	A	Wildtype	HEX™	≥ 24.20 – ≤ 33.20	
		G	Variant	FAM™	≥ 23.45 – ≤ 32.45	
	c.2846A>T	A	Wildtype	ATTO™550	≥ 23.62 – ≤ 32.62	
		T	Variant	ROX	≥ 25.78 – ≤ 34.78	
Mix 4	c.868A>G	A	Wildtype	HEX™	≥ 22.68 – ≤ 31.68	
		G	Variant	FAM™	≥ 24.21 – ≤ 33.21	
	c.2279C>T	C	Wildtype	ATTO™550	≥ 22.95 – ≤ 31.95	
		T	Variant	ROX	≥ 23.80 – ≤ 32.80	

Sample Validity Criteria

Recommended sample validity criteria for QS5 Dx instrument are provided below. The suitability for the PCR instrument under use should be defined by the user.

It is recommended that, for each sample, the Ct values for the wild-type target (ATTO™550) are ≤ 33.5 in both PP Mix 1 (c.1679T) and PP Mix 4 (c.2279C).

If a sample does not meet the acceptable Ct specification, the sample is invalid and should be repeated. Since the root cause of sample invalidity could be due to presence of inhibitor or sample input above or below the assay working range, it is recommended that when re-testing invalid samples the user tests dilutions of sample above and below the initial sample input. For example, if a diluted 1% (v/v) whole blood sample initially gives an invalid result with the kit, the user should repeat testing with 2% and 0.5% blood inputs to determine whether insufficient sample or presence of inhibitor is likely to be the root cause of the invalid result.

Results Interpretation

Ct values for the variant allele signal (FAM™ or ROX) relative to wildtype allele signal (HEX™ or ATTO™550) for each variant should be used to support the research-based interpretation of sample genotype: homozygous wildtype, heterozygous (both alleles present) or homozygous variant.

To determine the genotype call for each variant, calculate the ΔCt using the equation below:

$$\Delta Ct = Ct(Wildtype) - Ct(Variant)$$

Important notes:

- Use Ct values from the same reaction mix and sample for both signals (wildtype and variant)
- If no amplification is detected for a target, assign a Ct value of 40
- If both wildtype and variant signals show no amplification for a given sample and variant, consider the results for that sample invalid

When using QS5 Dx instrument, ΔCt cut-off values presented in Table 8 may be used to guide genotype classification for research purposes only. Users of other instruments should determine the ΔCt cut-off for each variant empirically through testing known wildtype and heterozygous research samples for each variant.

Table 8: Recommended cut-offs for establishing the genotype call.

Variant (NM_000110.4)	ΔCt cut-off		
	Wildtype Homozygous	Heterozygous	Variant Homozygous
c.1905+1G>A	< – 2.13	≥ – 2.13 ≤ 1.80	> 1.80
c.1679T>G	< – 3.00	≥ – 3.00 ≤ 1.22	> 1.22
c.1129-5923C>G	< – 2.49	≥ – 2.49 ≤ 2.36	> 2.36
c.557A>G	< – 2.44	≥ – 2.44 ≤ 3.30	> 3.30
c.2846A>T	< – 3.56	≥ – 3.56 ≤ 0.32	> 0.32
c.868A>G	< – 4.93	≥ – 4.93 ≤ 0.83	> 0.83
c.2279C>T	< – 3.64	≥ – 3.64 ≤ 1.32	> 1.32

When analysing homozygous samples with the ExaccType DPYD Kit, low-level amplification of both variant and wildtype assays can be observed. Therefore, interpretation of genotype should be based on ΔCt values rather than amplification signals alone.

Performance Evaluation

Concordance

The concordance of the ExaccType DPYD Kit was demonstrated relative to gDNA from 29 cell line (Coriell Institute for Medical Research)[1] samples pre-characterised using research genotyping methods such as NGS, microarrays and qPCR, Seraseq DPYD DNA Mutation Mix (SeraCare, Cat. No. 0750-9502) and one synthetic DNA sample for each variant. Cell line DNA and Seraseq control was tested at 1.05 ng/reaction) and the synthetic DNA was tested at 1000 copies/reaction.

100% agreement with the expected genotyping call was obtained for all samples tested (Table 9).

[1] The following DNA samples were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research: NA06991, NA12004 and NA12248. The following DNA samples were obtained from the NHGRI Sample Repository for Human Genetic Research at the Coriell Institute for Medical Research: HG03196, HG03911, NA20797, NA20531, NA20515, NA19321, HG01914, NA20812, HG00185, HG02684, HG02645, HG03645, NA19921, NA19741, HG03771, HG02772, HG03716, NA20901, NA21114, NA21119, HG00118, HG00285, NA20362, HG00129, HG00332 & NA19207

Table 9: ExaccType DPYD Kit agreement with other research genotyping methods.

Variant (NM_000110.4)	N° Homozygous Wildtype samples tested	N° Heterozygous samples tested*	N° Homozygous Variant samples tested+	N° of correctly genotyped samples
c.1905+1G>A	24	5	2	31/31
c.1679T>G	27	3	1	31/31
c.1129-5923C>G	23	6	2	31/31
c.557A>G	24	6	1	31/31
c.2846A>T	24	6	1	31/31
c.868A>G	26	4	1	31/31
c.2279C>T	24	6	1	31/31

*One of which was the Seraseq DNA mutation mix, +One synthetic DNA per variant tested

Additionally, 100% agreement was obtained when comparing the result of 10 unique healthy donor EDTA whole blood sample results to matched extracted gDNA.

Troubleshooting

For information on troubleshooting, please contact support@biotype.de

Appendix A: Threshold Setting

The threshold should be set to a level higher than the baseline level of fluorescence but well below the plateau of the amplification plot. It must be positioned within the linear segment of the amplification curve, which indicates the detectable log-linear range of the PCR. For clarity, the threshold value should be established within the logarithmic view of the amplification plot to easily identify the log-linear phase of the PCR.

If multiple targets are used in the real-time experiment, the threshold needs to be determined individually for each target. Serial dilutions of template can be used to assess the correct threshold for your instrument, the threshold should be set across a range of template concentrations to determine the optimum threshold for each target (Figure 2).

Refer to the user guide of the qPCR platform in use for further information on threshold setting.

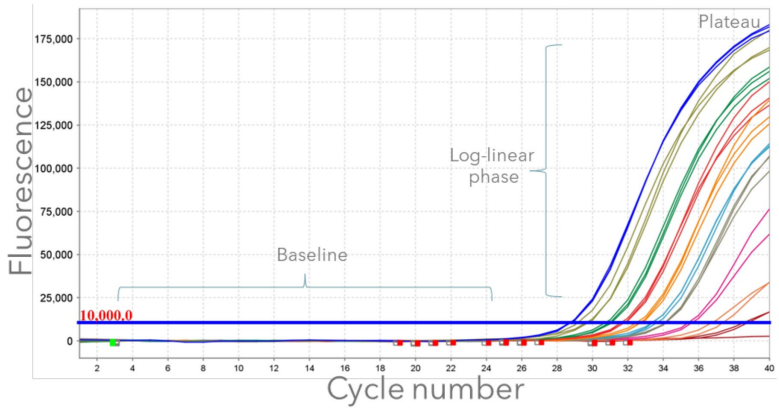


Figure 2: Example of a linear amplification across a range of template concentrations (indicated by the coloured lines). The PCR threshold (blue line) was set close to the inflection point (or the point at which a reaction reaches a level of fluorescence above the background level of the reactions). The PCR threshold was set to ensure that all amplification curves generate a cycle number (Ct value).

Technical Assistance



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Ordering information

Direct your orders via email to sales@biotype.de.

Product	Order number
ExaccType DPYD Kit	BT00092

Visit the BIOTYPE website at https://www.biotype.de/.

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Explanation of Symbols



Manufacturer



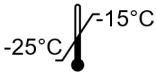
Batch code



Consult electronic user manual (eHB / eHandbook)



Use-by date



Temperature limit



Catalogue number

RUO

For Research Use Only. Not for use in diagnostic procedures.



Keep away from sunlight



Keep dry

Further marking used in this Handbook:

	Useful tips
	Attention, be sure to follow this notice!
blue underlined text	Links leading to external content like homepages, e-mail adresses
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