For further processing into IVD products and medical devices only.

AptaTaq Genotyping Master
5x concentrated

Version: 05
Content Version: January 2024

Master mix for genotyping and qPCR reactions.

Cat. No. 05 955 807 103	10 mL
Cat. No. 05 890 152 103	custom fill

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	AptaTaq Genotyping Master, 5x conc.	▪ Ready-to-use hot start PCR Mix. ▪ Contains AptaTaq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and 16 mM $MgCl_2$. *Contains all reagents needed for optimizing real-time genotyping or qPCR DNA detection except primers, probes, and the sample material.*	05 955 807 103	1 vial, 10 ml
			05 890 152 103	Custom fill

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	AptaTaq Genotyping Master, 5x conc.	Store at −15 to −25°C. For short-term storage, store at +2 to +8°C for up to 1 month. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Autoclaved reaction tubes for preparing PCR mixes and dilutions
- Standard benchtop microcentrifuge
- Thermal block cycler or real-time PCR instrument

For real-time PCR

- PCR reaction vessels, such as optical tubes or microplates
- Optical tube caps or self-adhesive foils
- Sequence-specific primers
- Template DNA
- Water, PCR Grade*

For carryover prevention (optional)

- Uracil-DNA Glycosylase*, or
- LightCycler® Uracil-DNA Glycosylase*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA, such as genomic or plasmid DNA, cDNA suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids, use:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation), or another standardized and automated extraction system,
- or a High Pure nucleic acid isolation kit (for manual isolation).
- Use up to 250 ng complex genomic DNA or 50 ng cDNA.

⚠ ***Store the template DNA in either Water, PCR Grade* or 5 to 10 mM Tris-HCl, pH 7.5 to 8.0. Do not dissolve the template in TE buffer since EDTA chelates Mg^{2+}.***

Control Reactions

Negative control

To detect DNA contamination, always include a negative control in each run. To prepare this control, replace template DNA with Water, PCR Grade*.

Primers

Use PCR primers at a final concentration of 0.1 to 1.0 µM. The suggested starting concentration is 0.3 µM when working with LightCycler® 480 ResoLight Assay and 0.9 µM when working with hydrolysis probes each.

⚠ ***Always use equimolar primer concentrations.***

ⓘ *The design of the PCR primers determines amplicon length, melting temperature, amplification efficiency, and yield. Primer design may also depend on the choice of PCR program (2-step versus 3-step protocol).*

ⓘ *Several programs for primer design are freely available or provided by the suppliers of real-time PCR instruments.*

ⓘ *If you already plan to evaluate your results by a hydrolysis probe (5' nuclease) assay later on, select primers with a Tm of +58 to +60°C.*

General Considerations

The optimal reaction conditions, such as concentration of template DNA and PCR primers, incubation temperatures and times, and cycle number depend on the specific template/primer system and must be determined individually.

Reaction volumes

Various reaction volumes of the AptaTaq Genotyping Master can be used. Refer to recommendations from the supplier of the instrument for suitable volumes and tubes and plates.

FRET-ROX reference dye

In principle, real-time PCR instruments, except the LightCycler® instruments offer two different modes:

- Detection of fluorescence in relationship to a reference dye, such as FRET-ROX.
- Detection of fluorescence alone.

The choice of mode depends on the instrument, for example, whether a channel for detecting the reference dye is available, and on the light source of the instrument (halogen versus laser). The AptaTaq Genotyping Master without FRET-ROX is proven to run on the LightCycler® 480 System*.

ⓘ *If you use the Bio-Rad iCycler iQ5 Real-Time PCR Detection System, use the AptaTaq Genotyping Master without FRET-ROX, and apply the External Well Factor Plate procedure for determining the well factors. For details on how to perform the External Well Factor Plate procedure, consult the iCycler iQ5 Real-Time PCR Detection System Instruction Manual.*

The AptaTaq Genotyping Master can also be used for standard PCR with commercially available thermal block cyclers following the supplier's recommended PCR protocols.

Prevention of Carryover Contamination

Uracil-DNA Glycosylase* (UNG) is suitable for preventing carryover contamination in PCR.

① This carryover prevention technique involves incorporating deoxyuridine triphosphate into amplification products, then pretreating later PCR mixtures with UNG.

② If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template. Since target DNA templates contain thymidine rather than uridine, it is not affected by this procedure.

dUTP is a component of the AptaTaq Genotyping Master.

Perform prevention of carryover contamination with LightCycler® Uracil-DNA Glycosylase*. Add 0.5 to 1.0 U per 20 µl PCR. Proceed as described in the Instructions for Use.

2.2. Protocols

Preparation of PCR master mix

For each 10 µl reaction, prepare the following reaction mix:

1. Thaw the solutions and, for maximum recovery of the contents, briefly spin vials in a microcentrifuge before opening.
   – Mix solutions carefully by pipetting up and down, then store on ice.

2. Prepare PCR primer solution, for example, 10 µM.

3. In a 1.5 ml reaction tube on ice, prepare the PCR mix for one 10 µl reaction by adding the following components in the order listed below:
   – To prepare the PCR mix for more than one reaction, multiply the amounts in the “Volume” column by z, where z = the number of reactions to be run plus one additional reaction.

Reagent	Volume [µl]	Final conc.
AptaTaq Genotyping Master, 5x conc.	2	1x
Forward primer	X	0.1 – 1.0 µM
Reverse primer	X	0.1 – 1.0 µM
Probe	X	0.1 – 0.4 µM
Water, PCR Grade*	add up to a volume of 8	–
Total Volume	**8 µl**	

4. Mix the solution carefully by pipetting up and down; do not vortex.
   – Pipette 8 µl PCR mix into each PCR reaction vessel or well of a PCR microplate, depending on your real-time PCR instrument.

5. Add 2 µl of template DNA, up to 250 ng genomic DNA or 50 ng cDNA.

   To determine the optimum amount of cDNA template in initial experiments, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel.

– Mix carefully by pipetting up and down.

6. Prepare the tubes or microplates for PCR according to the instructions supplied with your instrument, for example, seal tubes with optical tube caps or the plate with a self-adhesive foil.

Performing PCR

There are several different ways to program the PCR. Either two-step or three-step PCR programs will provide suitable experimental results. The amplicon should be short, approximately 150 bp, and the annealing/elongation temperature should be +60°C. For example, a typical PCR protocol is 40 cycles of +95°C/15 seconds, followed by +60°C/1 minute.

⚠ ***For best results, be sure the instrument is calibrated correctly.***

The following table shows an example of a standard two-step PCR protocol.

If you want to perform a fast qPCR protocol on an ABI instrument equipped with a FastPlate, apply the Hold Time given in brackets. This will reduce cycling time to about 1 hour.

1. Following the Operator's Manual of your instrument supplier, program the instrument with the following parameters:

Cycles	Analysis Mode	Target Temperature [+°C]	Hold Time	Remarks
1 (optional)	None	50	2 min	Only if UNG has been added for carryover prevention.
40	None	95	15 sec [10 sec(1)]	Amplification and real-time analysis.
	Quantification	primer dependent (typically 58 – 60)	60 sec [30 sec(1)]	

2. Place your tubes or plate in the instrument and start the reaction.

3. At the end of the reaction, follow instrument instructions for quantification and melting curve analysis.

(1) Hold Time when applying a fast PCR protocol.

3. Troubleshooting

Observation	Possible cause	Recommendation
No amplification detectable and no band in gel analysis.	Error in PCR program, for example, activation step omitted.	Adjust PCR Program.
	Pipetting errors, such as DNA not added.	Repeat experiment; check pipetting steps carefully.
	Amplicon too long.	Redesign primers.
	Inhibitory effects of impurities.	Repeat isolation of template.
	Bad primer design.	Redesign primers.
Fluorescence varies within a run.	Instrument not correctly calibrated.	Recalibrate instrument.
High background in the negative (no template) control.	Contamination present.	Remake or replace critical solutions, such as water.
		Clean lab bench.
		Use UNG to prevent carryover contamination.

4. Additional Information on this Product

4.1. Test Principle

How this product works

The AptaTaq Genotyping Master contains AptaTaq DNA Polymerase for hot start PCR to improve specificity and sensitivity of the PCR by minimizing the formation of nonspecific amplification products.

- AptaTaq Genotyping Master contains an optimized mixture of recombinant Taq DNA Polymerase and an aptamer oligonucleotide that reversibly binds to the enzyme. AptaTaq DNA Polymerase works similar to antibody-based methods with the benefit of being a reversible technology. Dropping the temperature below +55°C shuts off the polymerase activity; temperatures above +60°C fully activate the enzyme. The enzyme is active only at high temperatures, where primers no longer bind nonspecifically.
- AptaTaq DNA Polymerase does not require an activation step necessary for chemically modified hot start Taq DNA Polymerase, or extra handling steps typical of other hot start techniques, and thus is beneficial when total assay time is crucial.

4.2. Quality Control

Each lot is tested for performance in qPCR using three templates: a GC–rich template, a GC-poor template, and a long template, approximately 440 bp.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Updated regulatory disclaimer.

5.3. Trademarks

MAGNA PURE, APTATAQ, LIGHTCYCLER and RESOLIGHT are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

5.4. License Disclaimer

Consult product detail pages at **custombiotech.roche.com** for patent license limitations, if available.

5.5. Regulatory Disclaimer

For further processing into IVD products and medical devices only.

5.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.7. Contact and Support

For additional documentation such as certificates and safety data sheets, please visit **documentation.roche.com**.

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