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Aptamer Hot Start Master Mix

(Cat. No. A613, A614, A615, A615xl) rev. 01/2022

Description

Aptamer Hot Start Master Mix is an optimized ready-to-use solution containing Taq DNA Polymerase, aptamer anti-Tag, dNTPs, $MgCl_2$, dye, additives and stabilizers. Additives and the dye allow direct loading of the PCR-amplified samples into the gel without adding loading buffer. When compared to hot start master mix containing Taq polymerase-specific monoclonal antibody, aptamer-based hot start Master Mix has an advantage that (**1**) it is more resistant to elevated temperatures, (**2**) the inhibitory effect is reversible, and (**3**) master mix is more chemically defined. The master mix is not recommended for applications in which fluorescent DNA probes are used for detection amplified DNA (qPCR). It is ideally suited for routine PCR amplification of DNA fragments up to 5 kb. It is compatible with template DNA extracted with DEP-25 DNA extraction kit (Cat. No. D225-D227).

Hot start

- Aptamer Hot Start Master Mix contains DNA aptamer anti-Taqwhich binds reversibly to Taq DNA polymerase, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal cycling conditions allowing reactions to be set up at room temperature. The aptamer-based hot start does not require a separate high temperature incubation step to activate the enzyme. After PCR the aptamer regains its inhibitory activity.

Rapid samples preparation

- All components of the Aptamer Hot Start Master Mix are 2x concentrated, which facilitates rapid preparation of the PCR samples. Samples for PCR are prepared by mixing an aliquot of Aptamer Hot Start Master Mix with oligonucleotide primers, template DNA and H_2O (included).
- Aptamer Hot Start Master Mix is especially suited for routine analyses of large numbers of DNA samples. To 0.5 ml of the Master Mix in original tube, primers (e.g. 40 µl forward and 40 µl reverse) and PCR Ultra H_2O (380 µl) are added and mixed; the "armed" Mix can be stored at -20 ± 5°C. Immediately before use, the Mix is thawed and each 24 µl aliquot is mixed with 1 µl of the tested DNA template and PCR is performed.

Direct loading into the gel

- Aptamer Hot Start Master Mix contains additives and a dye which allows direct loading of the sample after PCR into the gel, without necessity to add loading buffer.
- Dye present in the Mix migrates in the agarose gel in front of the primers and therefore does not interfere with quantification of the PCR products. The dye and other additives have no effect on DNA amplification during PCR.

High efficiency and specificity

- Master Mix allows highly sensitive and specific amplification of corresponding DNA fragments from genomic DNA or from cDNA obtained by reverse transcription; it possesses $MgCl_2$ at a concentration suitable for most of PCRs.
- Anti-Taq aptamer significantly reduces production of nonspecific PCR products.

Technical data

Components and basic packaging

- 1 tube with 0.5 ml Aptamer Hot Start Master Mix (for 40 reactions, 25 µl each).
- 1 tube with 1.5 ml PCR Ultra H_2O (in basic package).

Composition

- Aptamer Hot Start Master Mix contains: 150 mM Tris-HCl, pH 8.8 (at 25°C), 40 mM $(NH_4)_2SO_4$, 0.02% Tween 20, 5 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP, 100 U/ml Taq DNA polymerase, DNA aptamer anti-Taq, dye, stabilizers and additives.

Storage

- At temperature -20°C ± 5°C. Material can be repeatedly defrosted. For short period of times (up to 3 days) material can be stored at up to 30°C. This allows its sending at ambient temperature (nature friendly).

Purity and quality control

- Purity of Taq DNA polymerase is verified by SDS PAGE, only one band of 94 kDa is observed in Coomassie blue stained gel. Material is free of nucleases.
- Each batch of the Master Mix is tested for amplification of a single copy gene in genomic DNA.

Protocol

Suggested basic protocol for PCR amplification using Aptamer Hot-Start 2x Master Mix

1. In a thin-walled PCR tube the following components are mixed:

Volume*	Reagent	Final concentration
12.5 µl	Aptamer Hot Start M. Mix	1x M. Mix (75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2.5 mM $MgCl_2$, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 200 µM dTTP, 50 U/ml Taq DNA polymerase, Aptamer anti-Taq, stabilizers and additives)
1 µl	5'-primer	0.1 - 1 µM (~ 20 bases in length)
1 µl	3'-primer	0.1 - 1 µM (~ 20 bases in length
1 µl	Template DNA	
9.5 µl	PCR H_2O	to a final volume 25 µl

*Different volumes can be used, but Master Mix should be finally diluted twice.

2. Mix gently and briefly centrifuge.

3. Add ~20 µl of PCR oil (Cat. No. P043) to prevent evaporation (oil is not required if thermal cycler with a heated lid is used).

4. Perform PCR under conditions optimized for the primers used. Common cycling parameters are:

	Temperature	Time	Number of cycles
Initial denaturation	94°C	1 min	1
Denaturation Annealing of primers Extension	94°C 55-68°C [1] 72°C	15 s 15 s 1 min per 1 kb	25-35
Final extension	72°C	7 min	1
Cooling	22°C		

[1] Should be determined experimentally; usually 5°C below melting temperature of the primers.

5. Amplified DNA can be directly loaded into agarose gel without adding loading buffer.

Cat. No.	Product name and specification	Quantity
A613	Aptamer Hot Start Master Mix (1x)	40 reactions
A614	Aptamer Hot Start Master Mix (5x)	200 reactions
A615	Aptamer Hot Start Master Mix (25x)	1000 reactions
A615xl	Aptamer Hot Start Master Mix (100x)	4x 1000 reactions