HS qPCR Apta-Mix

HS qPCR Apta-Mix (Probe, 2x) is a master mix kit containing a hot start polymerase and all other components necessary for PCR, ultra-pure dNTPs included. (Probe, DNA template and primers are not included)

This kit is perfectly appropriated for probe based real time PCR (qPCR) and possess aptamer hot start properties, suppressing any enzymatic activity until activation.

Thus, qPCR preparation at room temperature is feasible. Polymerase activation is carried out by heating at 95 °C for 2 minutes.

As the number of pipetting steps are reduced, the risk of cross-contamination is also diminished.

The optimal combination between polymerase and reaction buffer together with a robust aptamer inhibition diminish effectively nonspecific amplification and improve the yield of specific PCR products.

I. Components

	100 react.	500 react.
HS qPCR Apta-Mix (Probe, 2x)	1 x 1.25 ml	5 x 1.25 ml
Art. No.	3010.100	3010.500

II. Storage and stability

HS qPCR Apta-Mix (Probe, 2x) master mix should be stored at -20 °C.

*Note: **HS qPCR Apta-Mix (Probe, 2x)** master mix is stable for at least 12 months.*

III. HS qPCR Apta-Mix preparation

The HS qPCR Apta-Mix (Probe, 2x) is delivered ready-to-use and contains all components necessary for PCR, ultra-pure dNTPs included. (Probe, DNA template and primers are not included)

*Note: Multiple freeze/thaw cycles hasten ready to use master mix **HS qPCR Apta-Mix** degradation. Make aliquots if necessary. Ready to use **HS qPCR Apta-Mix (Probe, 2x)** can be stored at 4 °C for a period of one week.*

IV. Reaction mixture

Recommendations

a) Template DNA

Amount and quality of template DNA significantly affect PCR results. Too much template DNA raise the risk of non-specific amplicons whereas too little amount of template DNA lower accuracy. Therefore we recommend using in case of genomic DNA 1 – 300 ng template DNA per reaction and in case of plasmid or viral DNA 1 ng – 1 pg template DNA per reaction.

Template DNA quality can be improved by a preliminary purification step (ethanol precipitation, purification kit, etc.).

b) Primers and probe

Primer concentration of 0.05 – 1 µM should be preferred. Too high primer concentration can generate mismatches. Ideally maintain GC content at 40 – 60 %. Avoid complementary intra/inter primer sequences, otherwise secondary structure, like hairpins, or primer dimerization can arise. For optimal PCR conditions, forward and reverse primer should have similar (+/- 5°C) melting temperature (T_m).

For probe we recommend using a concentration of 0,05 - 0,3 µM. Avoid too long probes at risk to reduce hybridization specificity. Increase efficiency in reducing distance between primers 3’ end and probes 5’ end. Ensure that melting temperature (T_m) of the probe is 6 – 10 °C higher than the primers melting temperature.

Note: NCBI provides free of charges a primer design software (Primer-Blast).

c) $MgCl_2$

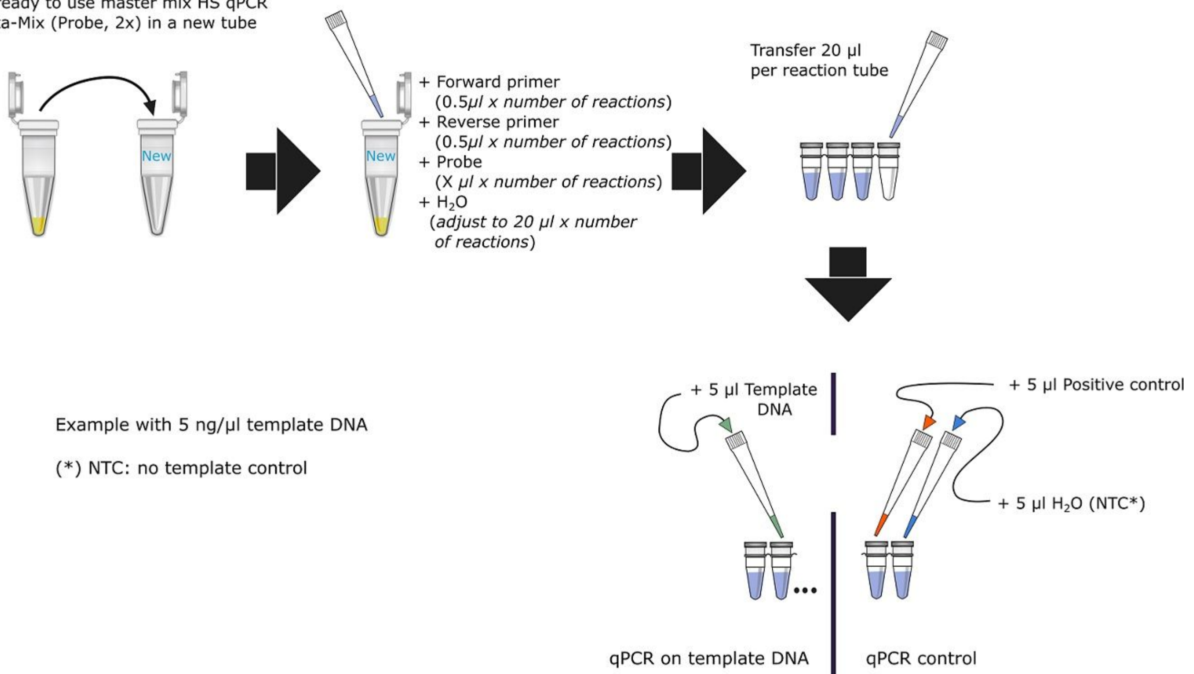
$MgCl_2$ concentration of **HS qPCR Apta-Mix (Probe, 2x)** has been established to fit in most situations. Nevertheless, it is possible to increase $MgCl_2$ concentration if appropriate.

Components	Volume	Final concentration
HS qPCR Apta-Mix (Probe, 2x)	12,5 µl	1x
Forward primer (10 µM)	0,5 µl	0,2 µM (1)
Revers primer (10 µM)	0,5 µl	0,2 µM (1)
Probe	x µl	0,2 µM (2)
Template DNA	y µl	1 ng < genomic DNA(3) < 300 ng
Sterile distilled water	Adjust to 25 µl	

(1) Optimal primer concentration range (0,05 à 1 µM)

(2) Optimal probe concentration range (0,05 à 0,3 µM)

(3) For viral or plasmid DNA use 1 ng - 1pg template DNA.



V. PCR settings

a) Denaturation and activation

The provided polymerase is inhibited by aptamers so that any enzymatic activity can take place before initial denaturation. Polymerase is activated by a 2-minute heating step at 95 °C.

Recommended denaturation time per amplification cycle is 15 seconds, adaptable according to the template DNA characteristics and thermocycler.

b) Annealing and extension

Optimal annealing temperature is generally 3 – 5 °C under primers melting temperature (T_m)

The approximate T_m can be calculated with the following formula:

$$T_m\ (°C) = 4 \times (G + C) + 2 \times (A + T)$$

Recommended annealing time per amplification cycle is 60 seconds, adaptable according to the template DNA characteristics and thermocycler.

Example of a 2 step PCR protocol:

	Temperature	Time	Cycles
Activation (initial denaturation)	95 °C	2 min.	1x
Denaturation	95 °C	15 sec. (1)	25 - 40 cycles
Annealing/Extension	60 °C (2)	60 sec. (1)	

(1) Adapt, if necessary, according to the template DNA characteristics and the thermocycler properties.

(2) Adapt according to primers T_m.

Example of a 3 step PCR protocol:

	Temperature	Time	Cycles
Activation (initial denaturation)	95 °C	2 min.	1x
Denaturation	95 °C	15 sec. (3)	25 - 40 cycles
Annealing	55 °C – 68 °C (4)	30 sec. (3)	
Extension	68 °C – 75 °C (4)	40 sec. (3)	

(3) Adapt, if necessary, according to the template DNA characteristics and the thermocycler properties.

(4) Adapt according to primers T_m.