A&A BIOTECHNOLOGY
innovating life science

3color Sensitive RT HS-PCR Mix SYBR®

High specificity ready-to-use mix for real-time Hot Start PCR with SYBR® Green. Dedicated for white reaction tubes and plates.

version 0217

250 reactions in 20 µl

Cat. # 2000-250SM

2500 reactions in 20 µl

Cat. # 2000-2500SM

A&A BIOTECHNOLOGY, Aleja Zwycięstwa 96/98, 81-451 Gdynia, Poland
tel: +48 58 6982194, +48 883 323 761, fax: +48 58 6228578
VAT PL5840254403
info@aabiot.com www.aabiot.com

Mix Contents

Component	250 rxns	2500 rxns
3color Sensitive Mix Sybr	2 x 1.25 ml	20 x 1.25 ml
Hot Start *Taq* DNA polymerase, 0.1 U/µl 5 mM $MgCl_2$ dNTPs (dATP, dCTP, dGTP, dTTP), 0.5 mM each dNTPs 2x reaction buffer with SYBR® Green pink dye		
40x Turquise Sample Buffer	1 x 1.25 ml	10 x 1.25 ml
Nuclease-free water	2 x 1.5 ml	20 x 1.5 ml

All solutions should be thawed throughly on ice, gently vortexed and briefly centrifuged prior use !

Store at -20 °C

Repeated freeze-thaw cycles may cause a slight decrease in fluorescence.

Remarks:
Product is recommended for R&D use only.
SYBR® is registred trade mark of Molecular Probes, Inc.

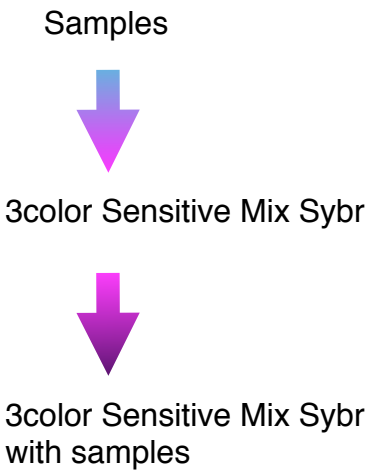
A&A Biotechnology provides one year guarantee on this mix.
The company does not guarantee correct performance of this mix in the event of:

* not adhering to the supplied protocol
* not recommended use of equipment and materials
* the use of other reagents than recommended or which are not a component of the mix
* the use of expired or improperly stored reagents

Description

3color Sensitive RT HS-PCR Mix SYBR® is a 2x concentrate solution of recombinant Hot Start *Taq* DNA polymerase blocked by a specific monoclonal antibody, PCR buffer with SYBR® Green, magnesium chloride, dNTPs and all other components required for PCR, except DNA templates and primers. The mixture contains also the pink dye therefore the post-PCR sample can be loaded directly onto an agarose gel and separated by gel electrophoresis. The premixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible qPCR.

3color Sensitive RT HS-PCR Mix SYBR® is supplemented with the inert pink dye and separate Turquise Sample Buffer that contains a turquise dye. Mixing both components in a qPCR reaction turns the solution violet. This provides a visual aid when pipetting and decreases the risk of errors during reaction setup, especially when using white reaction tubes. The dyes do not affect the specificity or sensitivity of qPCR assays.



An inert pink dye helps keep track of pipetting of the master mix into the reaction wells. It is easy to monitor which wells in a PCR plate are empty and which ones already contain the pink master mix. The maximum absorbation of the pink dye is 570 nm.

40x Turquise Sample Buffer is provided with 3color Sensitive RT HS-PCR Mix SYBR®. It is used to color and track the samples. When using the pink master mix the PCR reaction mix is pink before a sample is added. After adding the sample the reaction mix turns violet, making it easy to track the pipetting of samples. The buffer is provided as a 40x concentrate and used in 1x concentration in the final reaction. Using the Turquise Sample Buffer is optional. The maximum absorption of the turquise dye is 615 nm.

Recommended ROX mixtures

thermocycler	HiROX	
	per 20 µl	per 1.25 ml of 2x master mix
Applied Biosystems: 7300, 7900HT, 7900 HT Fast, OneStep™, OneStepPlus™, ABI PRISM® 7000, 7700	1 µl	30–50 µl
thermocycler	**LoROX**	
	per 20 µl	**per 1.25 ml of 2x master mix**
Applied Biosystems: 7500, ViiA™ 7 Stratagene: Mx3000P, Mx3005P, Mx4000	0.3–0.6 µl	30–50 µl
not required of ROX		
Thermo Scientific: PikoReal Real-Time PCR System Bio-Rad: iCycler® iQ, MyiQ™, Opticon®, CFX 96, CFX 384 Roche: LightCycler® 480, LightCycler® 2.0 Corbett: Rotor-Gene™ 3000, 6000 Eppendorf: MasterCycler™ ep realplex Cepheid: Smart Cycler®		

Design of assay

cDNA

For the first strand cDNA synthesis we recommend:
TranScriba Kit, cat. # 4000-20, 4000-100

The volume of cDNA added (from the RT reaction) to the qPCR reaction with 3color Sensitive RT HS-PCR Mix SYBR® should not exceed 10% of the final reaction volume. If high-abundance genes are to be detected, we recommend preparing a dilution of cDNA template prior to qPCR for the most accurate results. Then add the diluted cDNA up to 10% of qPCR volume.
If using the 40x Turquise Sample Buffer (optional), add 5 µl of this buffer to 20 µl cDNA synthesis reaction, then use 2.5 µl of the mix (this will compromise 2 µl of cDNA and 0.5 µl of Turquise Sample Buffer) into a 20 µl qPCR reaction.

DNA

Genomic DNA up to 200 ng and plasmid DNA up to 10 ng can be used in a 20 µl qPCR reaction with 3color Sensitive RT HS-PCR Mix SYBR®. Note that the plasmid copy number in 1 µg of plasmid DNA equals to 9.1 x 10^{11} divided by the plasmid size in kilobases.

In case of using the 40x Turquise Sample Buffer (optional), add buffer to samples in a concentration that will yield 1x final reaction volume. For example, if 5 µl of sample is to be used in a 20 µl reaction volume, add 40x Turquise Sample Buffer to obtain 4x buffer concentration in sample for 1x buffer concentration in the final reaction. A 4x Turquise Sample Buffer stock could be prepared by diluting 10 µl of 40x Turquise Sample Buffer with nuclease-free water to 100 µl.

Turquise Sample Buffer concentration in sample, when different amount of sample is to be used in a final qPCR reaction of 20 µl

Sample volume to be added to a qPCR reaction (20 µl)	Turquise Sample Buffer concentration needed in sample	Volume of 40x Turquise Sample Buffer in 100 µl of sample, giving the final concentration needed in sample
1 µl	20x	50 µl
2 µl	10x	25 µl
2.5 µl	8x	20 µl
3 µl	6.7x	16.7 µl
4 µl	5x	12.5 µl
5 µl	4x	10 µl
6 µl	3.3x	8.4 µl
7 µl	2.9x	7.2 µl
8 µl	2.5x	6.3 µl

Protocol

Reaction setup

1. Gently vortex and briefly centrifuge all solutions after thawing.

2. Calculate all components required for the appropriate qPCR volume.

Components (in order of addition)	10 µl rxns	20 µl rxns	50 µl rxns	Final concentration
3color Sensitive Mix Sybr *	5 µl	10 µl	25 µl	1x
10 µl Forward Primer	0.3 µl	0.6 µl	1.5 µl	0.3 µM **
10 µl Reverse Primer	0.3 µl	0.6 µl	1.5 µl	0.3 µM **
Template DNA (including 40x Turquise Sample Buffer, optional)	X µl	X µl	X µl	do not exceed 10 ng/µl in the final reaction
Nuclease-free water	up to 10 µl	up to 20 µl	up to 50 µl	

* provides a final concentration of 2.5 mM $MgCl_2$
* a final primer concentration of 0.3 µM is optimal in most cases, but may be individually optimized in a range of 0.05 µM to 0.9 µM

3. Prepare the reaction master mix by adding the 3color Sensitive Mix Sybr, nuclease-free water and primers (not included) for each qPCR reaction to a tube at room temp.

4. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.

5. Add template DNA (≤ 200 ng/reaction) to the individual PCR tubes or plates containing the master mix.

   NOTE: for two-step RT-qPCR, the volume of the cDNA added from the RT reaction should not exceed 10% of the final qPCR volume.

6. Gently mix the reactions without creating bubbles (do not vortex). Centrifuge briefly if needed. Bubbles will interfere with fluorescence detection.

7. Program the thermal cycler according to the recommendations below, place the samples in the cycler and start the program.

Thermal cycling conditions

Thermal cycling can be performed using a three- or two-step cycling protocol.

Three-step cycling protocol

Step	Temperature	Time	No. of cycles
initial denaturation	95 °C	5 min *	1
denaturation	95 °C	15 s	40
annealing	60 °C	30 s	
extension	72 °C	30 s	

* the time required to activate of Hot Start *Taq* DNA polymerase

Date acquisition should be performed during the extension step.

Two-step cycling protocol

Step	Temperature	Time	No. of cycles
initial denaturation	95 °C	5 min *	1
denaturation	95 °C	15 s	40
annealing/extension	60 °C	60 s	

* the time required to activate of Hot Start *Taq* DNA polymerase

Date acquisition should be performed during the annealing/extension step.

Optional steps

Melting curve analysis may be performed to verify the specificity and identity of the PCR product. Primer-dimers may occur during PCR if the primer design is not optimal. The dimers are distinguished from a specific product by a lower melting point.

Agarose gel electrophoresis of PCR products. When designing a new assay it is recommended to verify the PCR product specificity by gel electrophoresis, as melting temperatures of a specific product and primer dimers may overlap, depending on the sequence composition.

NOTE: The sample contains the dye therefore it can be loaded directly on the gel.

Safety information

All components from this kit are non-hazardous