-Datasheet-

BIO-Star Universal-Flex qPCR-Mastermix (2X) SYBR Blue

qPCR Mastermix with a novel ROX-alike composition

Cat. No. Y220, 2 x 1 ml

Features:

- A novel passive ROX alike reference dye. BIO-Star Universal-Flex Mastermix is compatible with all common PCR-Platforms (no ROX, low ROX of high ROX, see Combability list)
- Enzyme with hot start capability increases reaction specificity and sensitivity
- Hot Start Taq DNA polymerase with monoclonal antibodies
- High selectivity and reaction yield
- The mix is featured by a non-fluorescent blue dye for easy pipetting and visual control

Applications:

- Real-time PCR with intercalating dye SYBR Green I (valid for all ROX concentrations)
- Conventional PCR (cDNA, plasmid DNA, Lambda DNA)
- High-throughput PCR

Description:

The Mastermix contains all components all reagents required for qPCR (except template and primers) The SYBR Green I dye binds to double-stranded DNA formed during real-time PCR. At the same time, the product contains a special ROX Passive Reference Dye, which is suitable for use in all qPCR instruments, and there is no need to adjust the ROX concentration on different instruments.

- The mix is optimized for efficient and reproducible hot-start real-time PCR of genomic, plasmid and viral DNA samples.

- The solution contains substances that increase half-life and processivity of Taq DNA polymerase by enhancing its stability during PCR.

- smart components that influence primer annealing temperature and characteristics of template melting thus enabling to increase the specificity of PCR and use templates with complicated structure.

- The inert blue dye allows control when using multi-well plates. Use of the kit saves time and minimizes contamination risk due to reduced number of pipetting steps.

Components and Mixture

- 2X BIO-Star Universal-Flex qPCR-Mastermix SYBR Blue contains (2x1 ml):
The master mix is a ready-to-use solution including Hot Start Taq DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP) in an optimized PCR buffer system, SybrGreen I, novel psassive ROX alike as reference dye and blue inert-dye which does not interfere the PCR-reaction in a real-time Cycler.

- Water Mol.Bio Grade 2 x 1 ml

Storage and transportation: at -20 °C; not more than 50 thawing-freezing cycles. Shipping with blue ice or at room temperature

Storage terms: up to 18 months

Storage and Shipping Conditions

Ship with wet ice; store at -20°C, valid for 12 months.

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Assay Protocol / Procedures
Prepare the PCR reactions

Component	20 µl rxn	50 µl rxn	Final Concentration
2X Universal-Flex Blue SYBR Green qPCR Master Mix	10 µl	25 µl	1×
Forward Primer (10µM)[a]	0.4 µl	1 µl	0.2 µM
Reverse Primer (10µM)[a]	0.4 µl	1 µl	0.2 µM
Template[b]	Variable	Variable	as required
Nuclease-Free Water	Add to 20 µl	Add to 50 µl	

a: Usually, a good amplification effect can be obtained with the final concentration of 0.2 µM. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.2-1.0 µM.
b: The amount of template added varies depending on the number of copies of the target gene, and the appropriate amount of template addition is studied by gradient dilution. The best addition amount of template DNA in the 20 µl reaction system was less than 100 ng.
When the cDNA (RT reaction solution) of RT-PCR reaction was used as template, the addition amount should not exceed 10% of the final qPCR volume.

1. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
2. Transfer the appropriate volume of each reaction to each well of an optical plate.
3. Seal the plate with an optical adhesive cover, then centrifuge briefly to collect the contents at the bottom of each well and eliminate any air bubbles.
4. Program the thermal cycler according to the recommendations below, place the samples in the cycler and start the program.

A. Two steps method				
Stage	Step	Cycle number	Temperature	Time
Stage 1	Predegeneration	1	95°C	30 sec
Stage 2	Degeneration	40	95°C	15 sec
	annealing-extension		60°C	30 sec[a]
Stage 3	melting curve	1	Instrument default Settings	

B. Three steps method				
Stage	Step	Cycle number	Temperature	Time
Stage 1	Predegeneration	1	95°C	30 sec
Stage 2	Degeneration	40	95°C	15 sec
	annealing		55-65°C	10 sec
	extension		72°C	30 sec[a]
Stage 3	melting curve	1	Instrument default Settings	

a: If amplification specificity needs to be improved, two-step procedure or annealing temperature can be used;
To improve the amplification efficiency, a three-step procedure or extension time can be used.

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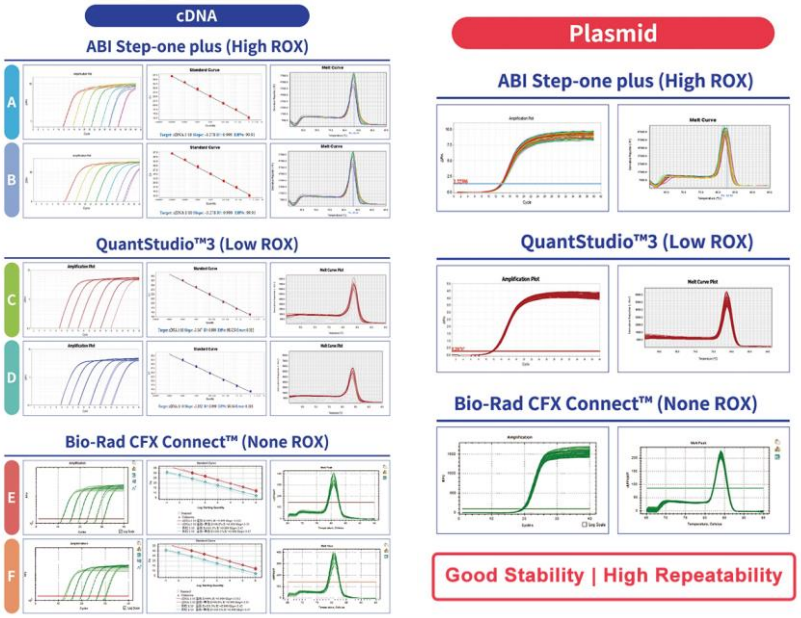
Compatible instruments / list

ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900 HT Fast, StepOne™, StepOne Plus™, 7500/7500 Fast, ViiA 7™,;
Analytik Jena: qTOWER series; qTOWER: LineGene series
Stratagene: Mx3000P®, 3005P™, 4000™;
Bio-Rad: CFX96™, CFX384™, iCycler
MiniOpticon™, Opticon®, Opticon 2,
Eppendorf: Realplex 2s, Mastercycler® ep,
IIlumina: Eco QPCR;
Cepheid: SmartCycler®;
QuantStudio™ series, PikoReal™ Cycler
Qiagen Corbett: Rotor-Gene® series;
Roche: LightCycler™ series;
Takara: Thermal Cycler Dice series;



Primer design principles

1. The length of amplification product is recommended to be between 80-300 bp;
2. Primer length: 18-25 bp;
3. The content of base G+C in primers should be between 40%-60%;
4. The Tm value difference between forward primers and reverse primers is less than 2°C and the Tm value between 58-62°C is the best;
5. Randomness of base distribution;
6. Primers had better not contain self-complementary sequences, otherwise they will form a secondary hairpin structure;
7. There should be no more than 4 complementary or homologous bases between two primers, otherwise primer dimer will be formed, especially complementary overlap at the 3' end;
8. The 3' terminal base of the primer is suggested to be G or C;
9. No other non-specific products were found in NCBI comparison results.

Ordering information.

Cat.-no	Description	Amount
Y220	BIO-Star Universal-Flex qPCR-Mastermix (2X) SYBR Blue	2x1 ml
Y220L	BIO-Star Universal-Flex qPCR-Mastermix (2X) SYBR Blue	10x1ml

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Trouble-Shooting

Problem	Possible cases	Solutions
No amplification curve appeared or CT value appeared too late	Annealing temperature is not optimal.	Optimize the annealing temperature in 3°C increments.
	Degradation of primers.	Check PCR primers for possible degradation on polyacrylamide gel.
	PCR inhibitors present in the reaction mixture.	Re-purify your template DNA.
	Primer design is suboptimal	Verify your primer design, use reputable primer design programs or validated pre-designed primers.
	RT-qPCR: inhibition by excess volume of the RT reaction.	Volume of RT reaction product added to qPCR reaction should not exceed 10% of the total qPCR reaction volume.
	Pipetting error or missing reagent.	Repeat the PCR reaction; check the concentrations of template and primers; ensure proper storage conditions of all reagents. Make new serial dilutions of template DNA or RNA.
Amplification signal in non-template control	DNA contamination of reagents.	Follow general guidelines to avoid carry over contamination; Discard reagents and repeat with new reagents.
	Primer-dimers.	Use melting curve analysis to identify primer-dimers by the lower melting temperature compared to amplicon
	RT-qPCR: RNA contaminated with genomic DNA	Design primers on intron/exon boundaries, treat RNA sample with DNase I, RNA free prior to reverse transcription.
The melting curve has multiple peaks	Primer design is suboptimal	Verify your primer design, use reputable primer design programs or validated pre-designed primers.
	Primer concentration is too high	Reduce primer concentration appropriately
	Genomic contamination in cDNA template	The extracted RNA solution is digested using DNA enzymes, such as dsDNase, to remove genomic contamination or to design transintron primers
Poor reproducibility of experiments	The error of adding sample is large	The use of accurate pipette, with high quality suction head accurate pipette; High dilution template, adding large volume template to reduce sampling error; The reaction volume of qPCR was enlarged
	The template concentration is too low	Repeat the experiment to reduce the dilution times of the template
	Temperature deviation at different locations of the qPCR instrument	Calibrate the qPCR instrument regularly
The amplification curve is not smooth	Fluorescence signal is too weak, produced after system correction	Ensure that the dyes premixed in the Master Mix are not degraded; Replace fluorescent signal to collect better qPCR consumables
Amplification curve breaks or slips	The template concentration was higher and the baseline endpoint value was greater than the CT value	The baseline endpoint (Ct value -3) was reduced and the data were reanalyzed
Amplification curves of individual Wells suddenly dropped sharply	There are bubbles in the reaction tube	Ensure that MIX is completely dissolved, and do not swirl and oscillate evenly; After the sample is added, the bubbles are removed by centrifugation with light elastic. The pre-denaturation time was extended to 10 min to remove the bubbles