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Lyo Universal Fast SYBR Mixture

Product Number: PCM64

Shipping and Storage

1. Storage and transportation at room temperature
2. Under normal temperature conditions, it can be stored for 12 months. If a longer period of time is needed, it can be stored at 2-8 ℃ for 24 months. Ensure that the aluminum foil bag is in a vacuum state during storage. After opening the eight row/frozen storage tube cap and glass bottle cap of this product, it should be re dissolved in nuclease free water within 1 hour (please refer to the instructions for specific operations). After re dissolving, it is a PCR premix that can be stored at -20℃ for 3 months and 4 ℃ for 2 weeks.

Specifications

24rxns 48rxns 100rxns

Description

Lyo Universal Fast SYBR Mixture is provided in freeze-dried form and is a specialized mixture for qPCR reaction using SYBR Green I chimeric fluorescence method. It can be transported and stored at room temperature before use. This mixture contains all the components required for PCR amplification, except for primers and DNA/cDNA samples. When used, it only needs to be re dissolved in water without nuclease. It has many advantages such as strong specificity, high detection sensitivity, and high amplification yield. Suitable for quantitative and qualitative analysis of DNA and cDNA samples, accurate detection can be performed within up to 6 logarithmic dynamic ranges. This mixture contains ROX Reference Dye, which is compatible with various qPCR instruments.

Protocol

1. Redissolution

   When in use, remove the cap of the eight row/freezer tube and the cap of the glass bottle, and add 10μL of nuclease free water to each reaction; Add 10μL of nuclease free water to each tube of the eight row for re dissolution, add 270μL of nuclease free water to each tube of the frozen storage tube for re dissolution, and add 1100μL of nuclease free water to each bottle of the glass bottle for re dissolution. After re dissolving, gently blow or vortex with a pipette to mix evenly. After re dissolving, it is 2x pre mixed liquid.
2. PCR reaction system
   - 2.1. After re dissolving the Lyo Universal Fast SYBR Mixture freeze-drying reagent as described above, the system can be directly configured. If using a pre mixed solution that has been re dissolved, take it out at -20 ℃ and thaw it completely at room temperature. After brief mixing and centrifugation, prepare the system.
   - 2.2. According to the table below, determine the total volume of appropriate reaction times and prepare PCR mixtures for all components except DNA/cDNA templates.

Reagent	20μl	Final Conc.
Lyo Universal Fast SYBR Mixture(2×Premixed liquid)	10μl	1×
Forward Primer,10μM	0.4μl	0.2μM
Reverse Primer,10μM	0.4μl	0.2μM
DNA template	Xμl	<1μg(total DNA)
Nuclease-Free Water	up to 20μl	

   - 2.3. After the configuration of the reaction system is completed, gently blow and mix with a pipette or mix with an instantaneous vortex, and collect the liquid at the bottom of the tube through brief centrifugation.
   - 2.4. During or after the configuration of the reaction system, when conducting equal volume sampling to qPCR tubes/96 well

plates, in order to obtain the best experimental results, it is necessary to ensure that the transfer volume is basically consistent during the operation process and minimize the generation of bubbles.

2.5. When adding DNA/cDNA templates to qPCR tubes/96 well plates, use a transparent cover with good transparency and no damage to seal, and use a fluorescent quantitative PCR transparent sealing film. Pay special attention to the sealing cover tightly and the edges and corners of the sealing plate to ensure complete sealing to prevent aerosol contamination caused by evaporation.

2.6. Before starting the machine, the qPCR tube/96 well plate (at 2500-3000rpm for 1 minute) needs to be centrifuged instantaneously to remove bubbles and collect all the liquid to the bottom.

3. PCR reaction program

3.1. Use the SYBR®Green Reagents "full channel scanning" mode setting on the fluorescence quantitative instrument. To obtain experimental results faster, the "Fast" fast program can be used according to the actual situation of the instrument (such as Applied Biosystems StepOnePlus, QuantStudio, 7500 and other fast program instruments).

Step	Temperature	Time	Cycles
Pre denaturation[1)]	95℃	5-30s	1
Denaturation[2)]	95℃	5-15s	40-45
Annealing/Extension[3)]	60℃	10-30s *	
	95℃	15s	
Melting curve[4)]	60℃	1min *	
	95℃	1s	

Note:1)The pre denaturation condition is suitable for the vast majority of amplification reactions, with a standard program of 30 seconds and a fast program of up to 5 seconds. If the template structure is complex, the pre denaturation time can be extended to 3 minutes to improve the pre denaturation effect.

2)Transmutation: standard program selection for 10 seconds; The shortest option for a fast program is 5 seconds.

3)Annealing/Extension: Standard program selection for 30 seconds; Quick program: For amplicons within 200bp, the minimum extension time can be set to 10s; When it exceeds 200bp, the recommended extension time is 30s.

4)Please set the melting curve analysis according to the recommended program of the fluorescence quantitative PCR instrument used. This program is based on the ABI-Q5 fluorescence quantitative PCR instrument as a reference setting.

5)*Set signal acquisition at the annotation point.

Result analysis

1. For basic information on real-time fluorescence quantitative PCR instrument data analysis, please refer to the user manual of the corresponding instrument.
2. After running, check the amplification curve to ensure that the baseline threshold is set within the range corresponding to the PCR index and above any background signal.
3. Amplification curve: The standard amplification curve is S-shaped.
   3.1. When the Ct value falls between 20-30, quantitative analysis is the most accurate;
   3.2. If the Ct value is less than 10, it is necessary to dilute the template and conduct the experiment again;
   3.3. When the Ct value is between 30-35, it is necessary to increase the template concentration or increase the volume of the reaction system to improve amplification efficiency and ensure the accuracy of the result analysis;
   3.4. When the Ct value is greater than 35, the detection results cannot quantitatively analyze the expression level of genes, but can be used for qualitative analysis.
4. Melting curve:
   4.1. The melting curve has a single peak, indicating good reaction specificity for quantitative result analysis; If there are double or multiple peaks in the melting curve, quantitative analysis cannot be performed.
   4.2. The melting curve shows a double peak, and it is necessary to determine whether the non target peak is primer dimer or non-specific amplification. If it is a primer dimer, it is recommended to reduce the primer concentration or redesign

primers with high amplification efficiency. If it is non-specific amplification, please increase the annealing temperature or redesign primers with higher specificity.

Primer Design Guidelines

1. The recommended primer length is around 25bp. The optimal amplification product length is 150bp, which can be selected between 100bp and 300bp.
2. The difference in Tm values between forward and reverse primers should not exceed 2℃. The optimal Tm value for primers is between 56℃ and 65℃.
3. The distribution of primer bases should be uniform, avoiding the occurrence of four consecutive identical bases, and the GC content should be controlled at around 50%. The last base at the 3 'end is preferably G or C.
4. It is best to avoid complementary sequences with more than 3 bases within the primer or between the positive and negative primers.
5. Primer specificity needs to be verified using the NCBI BLAST program. Avoid non-specific complementarity with more than 2 bases at the 3 'end of the primer.
6. The designed primers need to be tested for amplification efficiency, and only primers with the same amplification efficiency can be used for quantitative comparative analysis.