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AceQ™ qPCR

Probe Master Mix

YH Biosearch

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SHANGHAI YEHUA Biological Technology Co., Ltd.

Instructions

Version 1.2

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Catalog

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1/Product Abstract

This product is the specialized reagent for qPCR with probes. AceTaq TM HS DNA Polymerase activated on heat along with the most proper buffer optimized for qPCR, can restrain non-specific amplification efficiently, resulted in a much higher amplification efficiency, which makes it suitable for qPCR reactions with a high sensitivity. This product is convenient for use as a 2× Mix. With this product, a good standard curve can be achieved with this product in a broad quantitative area, the target gene can be quantified and tested accurately, the duplication will be good and the reliability will be high.

2/Product Composition

[a]Containing AceTaqTM HS DNA Polymerase，dNTP Mix，Mg2+.
[b]To revise flourescent signal errors between holes. Applying 50 × ROX Reference Dye 1 when using ABI 7900HT/ 7300 Real-Time PCR System or StepOne Plus ™ ; applying 50 × ROX Reference Dye 2 when using ABI 7500, 7500 Fast Real-Time PCR System or Stratagene Mx3000P; ROX is not necessary for Roche, Bio-Rad Real Time PCR.

Product Composition	Q112-01 (50 rxn/50 µl/rxn)	Q112-02 (200 rxn/50 µl/rxn)	Q112-03 (1,000 rxn/50 µl/rxn)
AceQ™ qPCR Probe Master Mix [a]	1.25 ml	1.25 ml × 4	
50 × ROX Reference Dye 1 [b]	50 µl	200 µl	Q112-02 × 5
50 × ROX Reference Dye 2 [b]	50 µl	200 µl	

3/ Preservation and Validity

This product should be preserved at -20℃; avoid multigelation during experiment. Subpackages of small dose are recommended if that is necessary.

4/Experiment Announcements (Please read carefully)

1)Avoid multigelation reducing enzyme activity and subpackages of small dose are recommended if that is necessary.
2)Turn upside down to blend Mix before using it. Do not vortex, in case the extra bubbles causes the misalignment of reaction system's volume and hence the influenced quantitative result. Blending mix can be used after being lightly centrifuged. Movement range should be slight and a second centrifuge is necessary if extra bubbles occur because of inappropriate operations.
3)Reaction system should be prepared in an ultra-clean bench, in case traces of aerosol in the air pollute this product because of its high testing sensitivity and lead into an experiment failure. Clean sterilization spears and reaction tubes are required during preparation; specialized pipettor is recommended if laboratory conditions permit, to avoid pollution. Pepittor with filter element are recommended.

5/Reference Implementation

Use AceQTM qPCR Probe Master Mix or qPCR Master Mix provided by Q company combined with ROX reference dye to do a real-time quantitative and PCR test on human GAPDH. Templates are 10 times diluted genomic DNA of Hela cell (104 to10 duplication). Testing machinetype is ABI StepOne PlusTM.
Note: 3 repeating reactions at least should be set for each concentration in actual applications (Quantitative Results Analysis requires at least 3 repetitions to be meaningful.)

1）qPCR reaction system preparation

AceQ™ qPCR SYBR® Green Master Mix	10	μl
Primer 1 (10 μM)	0.4	μl
Primer 2 (10 μM)	0.4	μl
TaqMan Probe (10 μM)	0.2	μl
50 × ROX Reference Dye 1	0.4	μl
Template DNA	5	μl
Sterile distilled water	Up to 20	μl

Note: Volumes of different composition can be adjusted according to following principles:
I Generally speaking, primer concentration of 0.2 μM leads to a better amplifications. When reaction performances are bad, primer concentration can be adjusted ranging from 0.1-1.0 μM.
II Probe concentration can be adjusted ranging from 50 nM-250 nM.
III qPCR has a super high sensitivity, accuracy of template volume added to build the reaction system has a great influence on the final quantitative result. Therefore, if equipment permits, 50 μl reaction system is recommended, and the templates should be diluted(to 5μl for example) before being added to the system to efficiently raise repeatability.
IV The volume of cDNA template should not be over 1/10 of the reaction volume.

2）qPCR reaction procedure set

Usually the reaction proceeds in two steps as following picture shows:
Anneal/Extension set on 60℃, Three steps procedure is also feasible for a quantitative test. Note: Since AceTaq™ HS DNA Polymerase needs to recover its enzyme activity over heat, please set PCR reaction predegeneration condition at least on 95℃ for 5 minutes. If the template contains high GC, predegeneration time can be extended to 10 minutes.

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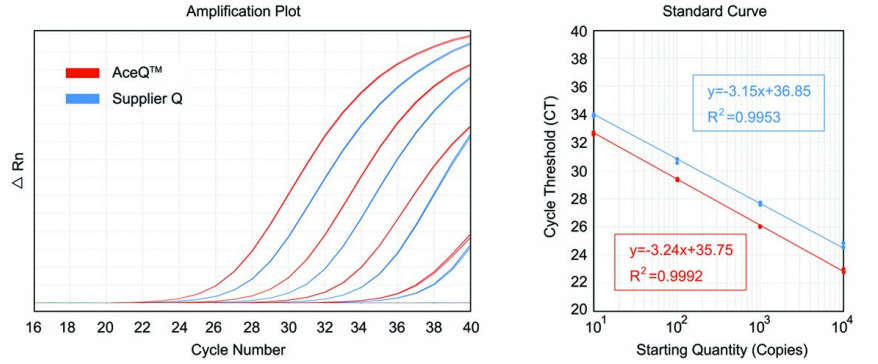
Two-step standard procedure:

Stage1 Predegeneration	Reps：1	95℃	5	min
Stage2 Circular reaction	Reps：40	95℃	10	sec
		60℃	30*	sec

*Extending time can be adjusted according to the time limit of Real Time PCR equipment to collect data: at least 30s when using ABI 7700 and 7900HT；31s at least when using ABI 7000 and 7300；34s at least when using ABI 7500；10s at least when using ABI StepOne PlusTM .

3）Confirm the Amplification curve(Figure on left) after the reaction, make a Standard Curve(Figure on right). Specificity of PCR amplification products can be confirmed with sepharose gel through electrophoresis.



Take AceQ™ qPCR Probe Master Mix(the red line) or quantitative PCR Master Mix provided by Q company(the blue line), along with ROX reference dye to do a qPCR test on human GAPDH. Amplification Plot shows that AceQ™ qPCR Probe Master Mix has a better amplification ability, a lower limit(left) and a good linear relation (right) within testing area.

6/Primer Design Guideline

1) Ideal length of primer is 17-25 mer. Either longer or shorter primer would affect the accuracy of quantitative results: too short a primer lower easily the amplification efficiency and too long a premier will result in a higher possibility of premier higher structure occurring.
2) GC content of primer should better be controlled within 40%-60%, best to be between 45%-55%.
3) Primer A、G、C、T should better be arranged balanced. Areas with high GC or TA should be avoided, especially the 3' end,. Areas with unbalanced GC must be avoided.
4) Primer designing should better avoid continuous structures of T/C or A/G.
5) The last 5 basic groups of the 3' end of premier should contain 2 G/C or less.
6) Both forward premier and reverse premier should be close to probe sequence, but cannot be overlapped.

7/TaqM an Probe Design Guideline

1) Probe sequence should be as close as possible to either forward or reverse premier but not overlapped.
2) Regular probe length is ranging from 18-40 mer.
3) Continuous same basic groups should be avoided, especially GGGG or more continuous G.
4) G basic group should be avoided on the 5' end of probes.
5) Anneal temperature of probes should be 65-67℃.
6) If polymorphic sites are concluded, they should be put in the middle of sequence.

8/FAQ & Solutions

1). Abnormal shape of amplification curve

a). Rough curve: weak signal, occurs on system's correction. A second test should be done with higher template concentration.
b). Fractured or gliding curve: high template concentration, the finishing end of base line is bigger than Ct. Reduce the finishing end (Ct- 4) of base line and analyses the data again.
c) Some curves plunge: reaction tube has gotten bubbles inside, and bubbles burst when temperature gets higher,only to give the equipment a plunging fluorescence value. Centrifugations should be paid attention to when dealing with templates; reaction tubes should be carefully checked before amplification reaction if there is any bubble left.

2). No amplification curve occurs after reaction

a). Not enough reaction cycles: usually reaction cycles are set to be 40. Too many cycles would increase more background signal to reduce data reliability.
b). To check if signal collection procedure is set: it is usually set at Anneal/Extension stage in two-step amplification procedure while at 72℃ Extension stage in three-step amplification procedure.
c). To confirm if the probe/primer is degraded: the integrity of the probe/primer unused for a long time should be tested through PAGE electrophoresis to avoid the possibility of primer's degradation.
d). Too low the template concentration: to reduce the dilution and repeat the experiment, usually begin with the highest concentration if the template concentration is unknown.
e). Template degradation: to re-prepare templates and do a second test.

3). Ct value occurs too late

a). Extremely low amplification efficiency: to optimize reaction conditions, try three-step amplification procedure or re-design a primer.

b). Low template concentration: to reduce the dilution and repeat the test usually begin with the highest concentration if the template concentration is unknown.

c). Template degradation: to re-prepare templates and a second test is necessary.

d) Too long PCR product: PCR products' lengths are usually designed between100 bp-150 bp.

e). PCR reaction inhibitors exist in reaction system: usually brought in along with the template, raise the dilution ratio or re-prepare templates for another test.

4). Obvious amplification in negative control

a). Reaction system or water is polluted: to replace with new Mix or water to repeat the experiment. Reaction system should be prepared on an ultra-clean bench to reduce aerosol pollution.

b). Primer dimer: amplifications in negative control are normal after 35 cycles.

5). Bad linear relation of standard curve in absolute quantitative tests

a). Sample addition error: to raise the dilution ratio and the sample volume.

b). Standard sample degradation: to re-prepare standard sample and repeat tests.

c). Too high the template concentration: to raise the dilution ratio of the template.

6). Bad repeatability in tests

a). Volume error in sample addition: to use pipette of better quality, expand reaction volume; to dilute the template at a high ratio and add the template dilution of big volume in reaction system .

b). Quantitative PCR equipment getting different temperatures at different places: regular calibrations on the equipment.

c). Too low the template concentration: the higher the dilution ratio is, the worse the repeatability. Lower the dilution ratio or enlarge the volume of adding sample.

7). If this product can be preserved at 4℃

a). No. Preservation at 4℃ would lead to a decline in products' activity.

b). Preservation at -20℃ is recommended for it would keep the products' activity for a long time.

c). Preservation in subpackages of small doses at -20℃ is recommended, for multigelation could reduce the products' activity.

8). Predegeneration time

This product is a Taq enzyme, hot-started on AceTaq™ HS DNA Polymerase with chemical modification. Predegeneration should be set at 95℃ for at least 5 minutes to release enzyme activity completely. Predegeneration time should be extended to 10 minutes if GC ratio is too high in the template.

9/Standards for Confirming the Validity of qPCR Reactions

1). Confirmation of linear relation and amplification efficiency:

Correlation indexes of standard curve (R2)＞0.98

Slope of standard curve between -3～-3.5

PCR amplification validity (E) between 0.9～1.2

2). Confirmation of repeatability: STD between reduplicate tubes ＜0.2

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