Taq-&GO™ Mastermix 5 x C

1000 reactions = 10x 1 ml

Cat. # 11EPTAG110

For research use only. Not for use in diagnostic procedures

Store 1 year at +4°C or 2 years at –20°C. Avoid successive freeze thaw cycles.

Taq-&GO™ is a 5 x C ready-to-use PCR mastermix containing all the components required for PCR (*Taq* DNA Pol, dNTPs, PCR buffer) except DNA template and primers. Taq-&GO™ exhibits the same specificity and efficiency as Qbiogene Molecular Biology's *Taq* DNA Pol and other components like dNTPs, when used separately.

Simply add 10 µl of Taq-&GO™ (5 x C) to a 40 µl volume containing DNA template and specific primers to achieve a 50 µl PCR reaction.

Final concentrations at 1 x C, are 1.5 mM $MgCl_2$ and 200 µM dNTPs.

By avoiding multiple pipetting, the risk of contamination and miscal-culation is greatly reduced. Using Taq-&GO™ Mastermix, avoids dilution of the traditional 5 U/µl *Taq* DNA Pol and eliminates thawing time.

A. Unit definition:
One unit of DNA polymerase is the amount of enzyme required to catalyse the incorporation of 10 nanomoles of nucleosides into a DE81 adsorbable product within 30 min at 74°C under assay conditions.

B. Absence of contamination:
The *Taq* DNA Polymerase used is a highly purified recombinant enzyme, free of any nickase, endonuclease, 3' exonuclease and ribonuclease.
All other components used in Taq-&GO™ (5 x C) Mastermix, are checked to be free of any endo or exonuclease.

C. Example of PCR assay on human genomic template:
This test is carried out as a quality control for every batch of Taq-&GO™ Mastermix and the results are compared to the traditional method using separate components.

A 400 bp region of the human β-globin gene was amplified using specific primers. PCR assays with decreasing amounts of DNA template were compared using either Taq-&GO™ 5 x C or the separate components (traditional method). PCR products were then analysed using agarose gel electrophoresis.

Assay conditions:

Taq-&GO™
Reaction volume: 50 µl
Taq-&GO™ (5 x C): 10 µl

Template DNA: 100 pg to 100 ng
Specific primers: 50 pmoles each

Traditional method
Reaction volume: 50 µl
Incubation buffer T. Pol (10 x C) with $MgCl_2$: 5 µl
Template DNA: 100 pg to 100 ng
Specific primers: 50 pmoles each
dNTPs: 10 nmoles each (200 µM final)
Taq DNA Pol: 1.5 U

PCR program:
(5' at 93°C) - [(1' at 93°C - 1' at 62°C - 2' at 72°C) x 37]
- [(1' at 93°C - 1' at 62°C - 10' at 72°C) x 1]

Results: Specific PCR products of 400 bp in length are obtained in all assays. Taq-&GO™ is as efficient as the traditional method whatever the quantity of DNA template used.

D. Repetitive PCR tests:
Repetitive PCR assays using Taq-&GO™ (5 x C) were regularly performed (as described previously) with various quantities of DNA template (human β-globin) to control the accurate rerproducibility of PCR efficiency.

E. Quantity and origin of DNA template:
The DNA template can be either plasmidic, or phagic, or genomic (total DNA extracted from cells), but also on a cDNA obtained from a mRNA by reverse transcription. A high level of DNA purification is not needed. There is no need to isolate the DNA sequences to be amplified, since the specificity of amplification is determined by the primers.

The amount chosen depends on the size of the PCR fragment and its origin:
- e.g. human genomic DNA amplifying 420 bp on a β-globin gene from 71.5 kb (total length):
1 pg to 200 ng of template = 6 fg to 1 ng of actual amplifiable DNA
- e.g. Lambda phage DNA amplifying 3.8 kb from 48.5 kb:
10 ng template = 800 pg of actual amplifiable DNA

When starting from a low quantity of DNA, the reaction remains in the exponential phase of duplication (10^5 molecules at the start generate 10^{12} molecules after 28 to 30 cycles). The larger the size of the fragment to amplify or the higher the amount of template, the sooner the reaction will be saturated in terms of duplication.

F. Reaction conditions:
Taq-&GO™ (5 x C) Mastermix is optimised for efficient and reproducible standard PCR. Nevertheless, each type of amplification needs an individual optimisation and depends on the type of template, the complementary sequences of the primers and the length of the amplified product. The following informations are provided as general advice.

G. PCR composition:
All reagents are added in sterile conditions, in a PCR microtube, preferentially on ice

Reaction volume…….50 µl
Taq-&GO™ (5 x C) Mastermix……. 10 µl
DNA template……..100 pg to 100 ng (according its origin)
Each primer……10 to 50 pmoles

PCR assays can be performed in a 20 µl volume, using 4 µl Taq-&GO™.

H. Guidelines for primer sequence design:
- One primer length depends upon its (A+T) content and the Tm of its partner
- The likelihood of annealing to sequences other than the chosen target, has to be very low.
- If "X" is the number of bases of a primer, 4^X (4 = the four nucleotides) is the size of the template where the primer sequence will be statistically present only once. The size of the human genome is about 3 x 10^9 bp, therefore the size of the primer (X) must be over 15 bases (over 20 bases for plants).
- Primers should have at least a minimal complementary sequence of 16 nucleotides to the template, for amplification on genomic DNAs of human and animals and 21-22 nucleotides for plants. That length should not exceed 28-30 bases.
- Addition of several mismatched bases (to introduce a restriction site) might occur at the 5' extremity of the primer
- A single mismatched base (introduction of a point mutation) can be located within or at any extremity of the primer sequence
- Addition of more than one mismatched base at the 3' end of the complementary primer will provoke a "breathing" effect preventing amplification
- Final base composition should always be 50-60% (G+C)
- Tm (melting temperature) of both primers should be equivalent and not exceed 70°C (4°C per G/C + 2°C per A/T)
- The annealing temperature depends directly on the length and the composition of the primers. One should aim at using an annealing temperature (Ta) about 5°C below the lowest Tm of the pair of primers to be used.

H. Guidelines for primer sequence design (continued):
- 3' ends of primers should not be complementary to avoid primer dimers
- Ability to form secondary structures such as hairpins should be avoided. If a particular position in the primer is required, but results in a secondary structure, try to move the primer slightly in either the 5' or the 3' direction on the complementary DNA template, until hairpins are avoided, or at least their Tm reduced.
- Primers with secondary structures can be shortened at either 5' or 3' extremities to make the hairpin unstable
- In all cases, when the hairpin cannot be avoided, the Tm of the hairpin must be significantly lower than the final annealing temperature, to enable denaturation of the secondary structure during the PCR process.

I. PCR program:
1. An initial denaturation step of 5 min at 93-95°C (not higher than 95°C) is recommended to ensure complete separation of the two DNA strands.
2. For subsequent cycles, a denaturation time of 30-60 sec at 93-95°C is sufficient for PCR fragments < 3-4 kb.
3. For longer PCR fragments than 4 kb (4 to 8 kb): as high temperatures (like 95°C) decrease the pH of reaction and thus, favour depurination, the time of denaturation must be reduced to 20 sec to ensure the amplification.
4. The annealing temperature depends directly on the primer sequences and the amount of sequence homology to the template. A simple formula to calculate the Tm is 4°C per G/C and 2°C per A/T and to place the annealing temperature 5°C below.
5. One consequence of too low a Ta, is a reduced yield of the desired product and appearance of non specific amplicons. One or both primers will anneal to sequences other than the true target, as internal single-base mismatches may be tolerated.
6. A consequence of too high a Ta, is a lower yield of product, as the likelihood of primer annealing is reduced and the initial template may not remain denatured.
7. For primers lengthened with non matched bases or containing point mutations, define the annealing temperature of the sequence complementary to the original template and use it for the first 3 to 5 PCR cycles. To define the annealing temperature for the last 20 or 30 cycles, consider the total sequence of the primers that will hybridise to the newly amplified fragments.
8. Most primers will anneal efficiently in 30 to 60 sec or less, unless Ta is too close to Tm, or primers are unusually long.

I. PCR program (continued):
9. For the elongation step at 72°C, 1 min per 1 kb is sufficient when amplifiable DNA is over 2 kb. Under 2 kb, this rule does not hold. Check in the user guide of your thermocycler, as the time needed for a specific length might change with the machine.
10. The number of cycles depends on the starting concentration of the target DNA. Between 20 to 37 cycles are recommended. Above 37 cycles, problems of specificity due to depletion of PCR components may occur. The PCR reaction is then subject to an attenuation in exponential rate of product accumulation in late stages of a PCR.
11. At the end of the PCR program, it may be desirable to perform a final extension for up to 10 min at 72°C. This reduces the number of incompletely amplified fragments.

J. Troubleshooting:
1. Little or no amplification observed:
- Check the Tm of the primers and their homologous sequences to the template.
- Verify the primers sequence and the absence of secondary structures, like primers dimers or hairpins.
- Lower the annealing temperature by 2°C to 5°C.
- If annealing temperature (Ta) is close to the elongation temperature, perform PCR by decreasing "Ta" every cycle.
- Raise initial denaturation temperature or increase its time
- Increase the elongation time.
- Increase the number of cycles by 5 (if <35).
- Vary the quantity of the template, and/or the primers.
- Consider the presence of inhibitory factors (e.g. NaCl, Mn^{2+}, SDS) introduced with the DNA template.

J. Troubleshooting (continued):

2. Multiple bands or smearing observed:
- Check the Tm of the primers and their homologous sequences to the template.
- Verify the primers sequence and the absence of secondary structures.
- Increase the hybridisation temperature of the primers by 2°C to 5°C to avoid non specific hybridisation.
- For primers lengthened with non matched bases or containing point mutations, check that the chosen hybridisation temperature is adapted
- Lengthen the homologous sequence of the primer to the template.
- When mismatched bases are added, increase the annealing temperature of the homologous sequences of the primers by 2°C to avoid non specific hybridisation of the non homologous sequences of the primers. And/or increase the annealing temperature of the complete primer by 2°C for the last 20-30 cycles.
- Reduce the number of cycles.
- Reduce the concentration of DNA template, of primers.

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