BioLit™ Taq Mix (2X)

Product Code : BPB005

Pack size – 1 ml (40 rxn), 5 x 1 ml (200rxn)

Description

Taq Mix (2X) is an optimized ready-to-use PCR mixture of Recombinant Taq DNA Polymerase, PCR Buffer, $MgCl_2$ and dNTP. PCR Mix (2X) contains all components for PCR, except DNA template and primers.

Applications

Standard PCR
DNA labeling
DNA sequencing
Numerous applications for which a high-quality thermostable DNA polymerase is required

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nM of dNTP into an acid-insoluble form in 30 minutes at 70°C using herring sperm DNA as substrate.

2X PCR Taq Mix

20 mM Tris-HCl (pH 8.0), 100 mM KCl , 3 mM $MgCl_2$, 400 µM dNTP, 0.1 U/µl Taq DNA Polymerase
Store at -20°C

Note

- PCR Mix is an optimized ready-to-use PCR mixture of Recombinant Taq DNA Polymerase which is the enzyme of choice for most PCR applications and the half-life of enzyme is >40 minutes at 95°C.
- The error rate of Taq DNA Polymerase in PCR is $2.2x10^{-5}$ errors per nt per cycle; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is $4.5x10^{4}$.
- PCR Mix accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- All solutions should be thawed on ice.
- Overlay the sample with mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid.
- Compatible with TA cloning – generates PCR products with 3'-dA overhangs.
- Recommendations with Template DNA in a 50µl reaction volume.

Human genomic DNA	0.1µg- 1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

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Basic PCR Protocol

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

Components	Volume	Final Concentration
2X PCR Taq Mix	25 µl	1 X
Primer mix (10 µM each)	4 µl	0.4 µM each
Template DNA	1–10 µl	n/a
Nuclease-Free Water	to 50 µl	n/a

2. Mix contents of tube and overlay with 50 µl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.
4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. Perform 25-35 cycles of PCR amplification as follows:

Step	Temperature	Duration
Denature	94°C	45 s
Anneal	55°C	30 s
Extend	72°C	1 min 30 s

6. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

Notes on cycling conditions

- Initial denaturation can be performed over an interval of 1-5 min at 95°C depending on the GC content of template.
- Optimal annealing temperature is 5°C lower than the melting temperature of primer-temperature DNA duplex. If nonspecific PCR products are obtained optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2°C.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.
- The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.

7. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.