EQ High Fidelity DNA polymerase kit, 250 u

Cat No: PD014S

Description:

EQ High Fidelity (HiFi) DNA Polymerase is a thermostable, chimeric DNA Polymerase designed especially for low-bias, high fidelity amplification of a broad range of amplicons. Due to its fusion with a DNA-binding domain, high-speed elongation and processivity is achieved. Fast amplification of amplicons >22 kbp never has been easier!

Unit definition:

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into an acid-precipitable form of DNA in 30 minutes at 72 °C under standard assay conditions.

Reagents supplied:

Cat no: PD014S		
Cat No	**Description**	**Tubes No.**
PD013-0.130	**EQ High Fidelity (HiFi) DNA polymerase 2 u/µl, 0.13 ml**	**1**
BR057-1.3	**5x EQ High Fidelity (HiFi) buffer (7.5 mM $MgCl_2$), 1.3 ml**	**2**
BR058-1	**25 mM $MgCl_2$, 1 ml**	**1**
BR059-1.3	**5M Betaine, 1.3 ml (after initial thawing betaine may be stored at 4°C for up to 6 months)**	**1**
BR028-0.275	**dNTPs mix 10mM each, 0.275 ml**	**1**
BR033-1.8	**Nuclease Free Water, 1.8 ml**	**2**

PCR Guidelines:

Prepare reaction mixtures in an area separate from that used for DNA preparation or product analysis. **Work on ice always.**

1. Thaw 5x EQ HiFi Buffer, dNTP mix and primer solutions.

A precipitate is often seen in the 5x EQ HiFi Buffer after thawing. It is recommended to completely thaw and thoroughly mix the buffer to ensure proper resuspension of precipitates.

2. Prepare a master mix according to Table 2. The master mix typically contains all the components needed for amplification except the template DNA.

It is important to add EQ High Fidelity DNA Polymerase last to prevent primer degradation caused by the 3'-5' exonuclease activity.

Version 5/jan-26

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PCR recommended reaction:

Components	25µl assay	Final Concentration/ Quantity (25µl assay)
5x EQ High Fidelity buffer (7.5 mM $MgCl_2$)	5µl	1x
dNTP mix 10mM each	0.5µl	0.2 mM each
10µM forward primer	0.5µl	0.2 µM
10µM reverse primer	0.5µl	0.2 µM
EQ High Fidelity DNA polymerase 2 u/µl	0.25µl (0.125-0.5)	0.5 u/25 µl reaction (0.25-1 units)
Optional 1. 25 mM MgCl2	0-3 µl	1.5-4.5 mM
Optional 2. Betaine (5M)	5-10 µl	1-2 M
Template DNA	Variable	100 pg-200 ng Human genomic DNA: 20 ng Bacterial DNA: 5 ng Plasmid DNA: 0.5 ng
Sterile ultrapure water	Up to 25 µl (*18.25-volume of template*)	N/A

Table 2. Recommended Reaction components

3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g., by pipetting the master mix up and down a few times.

4. Add template DNA to the individual tubes containing the master mix.

5. Program the thermal cycler according to Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

PCR recommended conditions:

Step	Temperature	Time
Initial denaturation	98°C[a]	2 min
18-35 cycles[d]		
Denaturation	98°C[a]	10-20 sec
Annealing	55-70°C[b]	20sec
Extension	72°C	10-60 sec[c]
Final extension	72°C	5 min
Hold	4°C	Indefinitely

Table 3. Three-step PCR program

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General instructions

a. **Denaturation time** may be increased if working with high complexity and high GC content templates (<60-65%). The GC content and distribution of the PCR target area may also affect the denaturation time.

b. The optimal Annealing temperature in PCR is usually 2-5 ◦C below the Tm of the primers used. Because of the high salinity of 5x EQ High Fidelity buffer, annealing temperature will likely be slightly higher than the lowest Tm (+1 to +3). An initial assay with a Ta gradient is suggested:

1.Ta=Tm-3

2.Ta=Tm

3.Ta=Tm+3

Depending on the result adjust at lower or at higher temperatures.

Betaine is a known additive in PCR reactions. It is used for enabling efficient melting of secondary structures of high GC content templates, hence the annealing temperature of the PCR reaction when betaine is used needs to be adjusted 2-4 ◦C below the respective annealing temperature of the same primer set previously estimated in reactions without betaine. It is strongly recommended in reactions for long amplicons (above 10 kbp) or when targeting regions with high GC content (above 60 %). As a general rule of thumb, when betaine is added at either final concentration (1-2 M) Ta is set at 4 ◦C below the lowest Tm of the primer set. Betaine at concentrations 0.5 -2 M, may also significantly improve the overall product yield of "easy" PCR setups.

c. Extension time:
If amplicon is <3 kbp
10-15 seconds/kbp for complex genomic targets (human genomic areas)
10 seconds/kbp for simpler targets (plasmid or λ DNA)

If amplicon is >3kbp
15-60 seconds/kbp for complex genomic targets (human genomic areas)
15-45 seconds/kbp for simpler targets (plasmid or λ DNA)
In case of amplicons above 15 kbp, increasing of template quantity and/or dNTPs concentration (up to 1.6 mM each) may increase PCR efficiency.

d. EQ High Fidelity DNA polymerase is 60x more reliable than Taq DNA polymerase. For site directed mutagenesis applications or when amplification of very long amplicons (amplicons >10 kbp) **with a minimum error rate** is required, **limit the cycles to 14-18.**

Other Quality Controls:

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Certified as DNase-RNase free using fluorescently labeled RNase and DNase probes. *E.coli* DNA contamination is tested through PCR for the detection of a 406 bp amplicon of *E.coli* 16S ribosomal DNA. Long PCR efficiency is verified by the amplification of a 22.2 kbp amplicon from λ DNA. No Nicking and Nuclease activity is detected.

Shipping
Shipped on blue ice or dry ice.

Storage conditions
Store at -20°C ± 5°C

Shelf life: 2 years

Important note when performing dilutions/ serial dilutions:

When preparing dilutions of either a DNA or an RNA sample at concentrations below 1 ng/µL, to use them as substrate for enzymatic reactions, it is advisable to prepare them with a "crowded" solution and not just water, especially if the long-term storage or several freeze-thaw cycles of the dilutions are required. DNA or RNA molecules tend to adhere to the micro-vials' walls, a fact that may greatly affect the outcome of molecular biology reactions below a certain concentration threshold. Different users, different dilution preparation events and different freeze-thaw cycles of the same dilution may lead to unreproducible results, even if you perform them from the same stock solution within a very short time span. This effect is more evident near the limit of detection of your assay. Our experience with many different types of substrates has shown that by using a 50 ng/µl standard carrier RNA solution you are able to avoid such predicaments (example 1). Any neutral macromolecule may be used as a crowding agent as long as it does not affect either the enzymatic activities or the analysis of the reactions, e.g sheered Salmon sperm DNA, tRNA solution, BSA.

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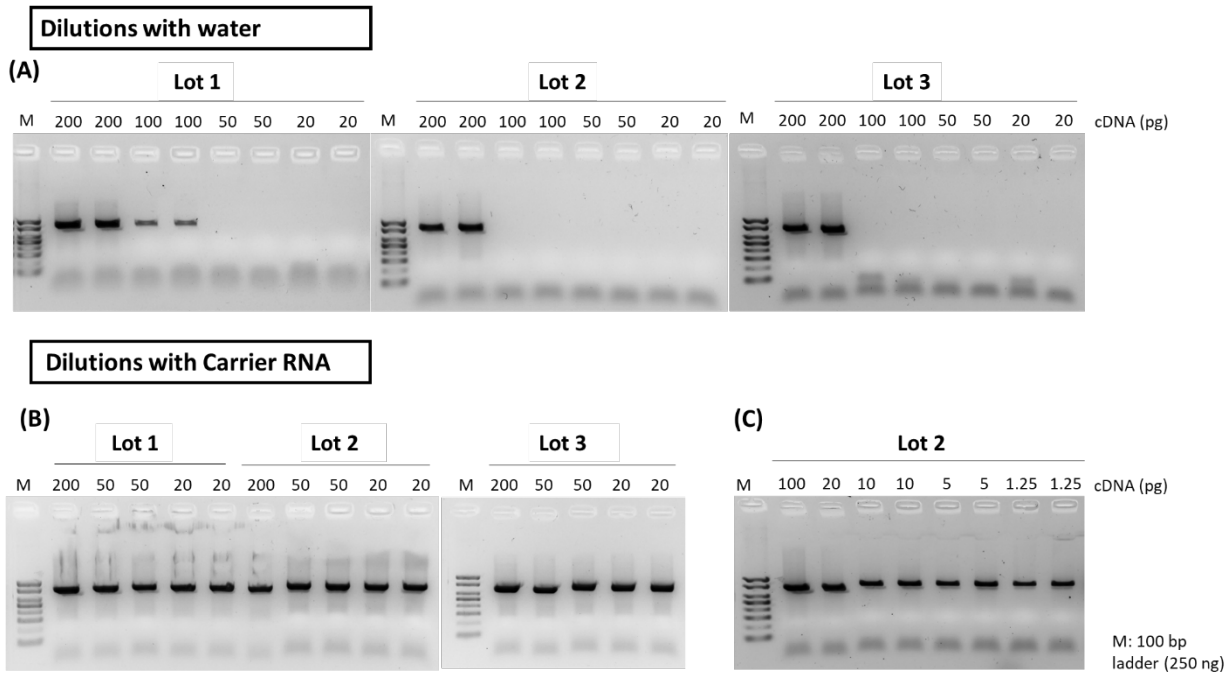

Example 1: Comparing dilutions of HeLa cDNA prepared either with water or with Carrier RNA solution as diluent, by end point PCR. Primers specific for the cDNA of GAPDH were used to check PCR efficiency of three different lots of EQ High Fidelity DNA Polymerase **A.** PCR results using as template cDNA dilutions prepared with water. Successful amplification is obtained in reactions where 100 pg of cDNA or above is added as template. **B.** PCR products from reactions containing template dilutions of the same cDNA prepared with Carrier RNA solution. Successful PCR amplification obtained in all reactions down to 20 pg/reaction. **C.** Results from one lot indicate that successful amplification may be obtained even in lower template quantities (100 to 1.25pg/reaction). cDNA was produced from 1 µg of HeLa total RNA, under standards conditions, using RN012S kit. *pg of cDNA definition*: even though the production yield of cDNA from total RNA using oligod$(T)_{18}$ does not lead to 1:1 mass conversion, we have made that assumption for simplification. That simplification does not affect the final interpretation of the result. E.g., from 1 µg of RNA, 1 µg of cDNA is produced, hence in a 20 µl standard Reverse transcriptase reaction the cDNA concentration is 50 ng/µl. The 1.25 pg equals to 4 x 10^4 dilution of the original cDNA sample.
All gels were prepared with the highly pure AG001-500 (Agarose for Molecular Biology, 500gr), due to its unique compatibility with Molecular biology analyses.

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