FFPE Direct PCR Kit

Quick preparation of template DNA from FFPE for PCR without DNA Isolation

Kit Contents

Cat. No.	TQ3000-01	TQ3000-02	TQ3000-03
Preps	20 preps	100 preps	500 preps
FP1 Buffer	2 ml	12 ml	60 ml
Proteinase K	3 mg	11 mg	5 x 11 mg
FP2 Buffer	1 ml	1 ml	5 x 11 ml
FP3 Buffer	2 ml	12 ml	60 ml
2 x PCR Master Mix	1 ml	2 x 1 ml	8 x 1 ml
Distilled water	2 ml	10 ml	50 ml

Shipping and Storage

The FFPE Direct PCR Kit is shipped at 2-8℃. 2 x PCR Master Mix should be stored at -20℃.

Product Description

The FFPE Direct PCR Kit contains all of the reagents required to rapidly extract and amplify genomic DNA from FFPE. Briefly, the DNA is extracted from a piece of FFPE, about 10 mg FFPE sample incubation in the Extraction Solution at 60°C for 30-60 minutes ,then put it in 90°C for 60 minutes. After an equal volume of the Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for PCR. An aliquot of the diluted extract is then combined with the 2 x PCR Master Mix and user provided PCR primers to amplify target DNA. 2 x PCR Master Mix is a 2 x Reaction Mix containing buffer, salts, dNTPs, and Taq DNA Polymerase. It is optimized specifically for use with the extraction reagents.

Protocol for FFPE Extraction

Prepare Proteinase K Solution: Add FP2 Buffer to dissolve Proteinase K and Store at -20°C.

TQ3000-01: Add 120 µl FP2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ3000-02: Add 550 µl FP2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ3000-03: Add 550 µl FP2 Buffer to each tube of Proteinase K, gently mix to dissolve Proteinase K.

1. **cut a approximately 10 mg FFPE sample into a 2 ml collection tube or suitable vessel**, cut off the Samples tiny and remove the paraffin wax as far as possible.
2. **approximately 10 mg FFPE sample add 95 ul FP1 Buffer and 5 µl FP2 to the collection tube.** Close the tube and vortex briefly. Make sure the sample is covered by the Extraction Solution.
3. Incubate at 60°C for 30-60 minutes.
4. Incubate at 90°C for 60 minutes.
5. **Add 100 µl FP3 Buffer and vortex to mix.**
6. Store the extraction at 2-8°C.

PCR Protocol

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may vary and must be individually determined.

1. **Thaw primer solutions.** Keep on ice after complete thawing, and mix well before use.
2. **Mix the PCR Master Mix by vortexing briefly.** It is important to mix the PCR Master Mix before use to avoid localized differences in salt concentration.
3. Prepare one of the following reaction mixes on ice: (For a 25 µl reaction volume)

Component	Volume	Final Concentration
2X PCR Master Mix	12.5 µl	1X
Upstream Primer, 10 µM	0.5 µl	0.1–1.0 µM
Downstream Primer, 10 µM	0.5 µl	0.1–1.0 µM
DNA Template	4 µl	<500 ng
Nuclease-Free Water to		25 µl

4. Gently mix the reaction and spin down in microcentrifuge.
5. **Set up program for a routine PCR reactions:**

Initial Denaturation	94-95°C for 1-5 min
25-40 cycles	94-95°C for 30 sec
	45-70°C for 10-30 sec
	72°C for X min(1min/kb)
Final extension	72°C for 7 min
Final soak	4-10°C

6. For a simplified hot start, proceed as described in step 7. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.
7. **Simplified hot start:** Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler. In many cases, this simplified hot start improves the specificity of the PCR.