Blood Direct PCR Kit

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

Kit Contents

Cat. No.	TQ2700-01	TQ2700-02	TQ2700-03
Preps	20 preps	100 preps	500 preps
Buffer NL (optional)	5 ml	30 ml	130 ml
BD1 Buffer	2 ml	12 ml	60 ml
Proteinase K	3mg	11mg	5 x 11mg
BD2 Buffer	1ml	1 ml	5 x 11ml
BD3 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 Blood Direct PCR Kit is shipped at 2-8℃. 2 x PCR Master Mix should be stored at -20℃.

Product Description

The Blood Direct PCR Kit contains all of the reagents required to rapidly extract and amplify genomic DNA from all kinds of blood samples, like human blood, pig blood, chicken blood, fish blood,frog blood etc. Briefly, the DNA is extracted from 100 ul erythrocyte without nuclear blood or 1-10 ul erythrocyte with nuclear blood sample incubation in the Extraction Solution at 56°C for 10 minutes ,then put it in 95°C for 5 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.

Prepare Proteinase K Solution: Add BD2 Buffer to dissolve Proteinase K and Store at -20°C.

TQ2600-01: Add 120 µl BD2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2600-02: Add 550 µl BD2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2600-03: Add 550 µl BD2 Buffer to each tube of Proteinase K, gently mix to dissolve Proteinase K.

Protocol for erythrocyte without nuclear blood

1. **Transfer 100ul Blood into a new tube. Add 250ul Buffer NL and invert the tube for 5 times.**
2. Centrifuge at 13,000g for 60 seconds. **Remove the supernatant. Add 95ul BD1 Buffer and 5 ul BD2 Buffer vortex 5 seconds to re-suspend the pellet.**
3. Incubate at 56°C for 10 minutes.
4. Incubate at 95°C for 5 minutes.
5. **Add 100 µl BD3 Buffer and vortex to mix.**
6. Store the extraction at 2-8°C.

Protocol for erythrocyte with nuclear blood

1. **Transfer 1-10 ul Blood into a new tube. Add 95ul BD1 Buffer and 5 ul BD2 Buffer vortex 5 seconds**
2. Incubate at 56°C for 10-30 minutes.
3. Incubate at 95°C for 5 minutes.
4. **Add 100 µl BD3 Buffer and vortex to mix.**

5. 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.