Tissue Direct PCR Kit

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

Kit Contents

Cat. No.	TQ2600-01	TQ2600-02	TQ2600-03
Preps	20 preps	100 preps	500 preps
AT1 Buffer	2 ml	12 ml	60 ml
Proteinase K	3mg	11mg	5 x 11mg
AT2 Buffer	1 ml	1 ml	5 x 11 ml
AT3 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 Tissue Direct PCR Kit is shipped at room temperature. 2 x PCR Master Mix should be stored at -20℃.

Product Description

The Tissue Direct PCR Kit contains all of the reagents required to rapidly extract and amplify genomic DNA from all kinds of animal tissue or Clinical samples, like mouse tails, ears, embryonic tissues or cultured mammalian cells, human hair, saliva, swab etc. Briefly, the DNA is extracted from a piece of tissue, about 5 mg tissue sample(or three human hair, one swab, 10 ul saliva) 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.

Protocol for Tissue Extraction

Prepare Proteinase K Solution: Add AT2 Buffer to dissolve Proteinase K and Store at -20°C.

TQ2600-01: Add 120 µl AT2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2600-02: Add 550 µl AT2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2600-03: Add 550 µl AT2 Buffer to each tube of Proteinase K, gently mix to dissolve Proteinase K.

1. **Cut a approximately 5 mg tissue sample(or three human hair, one swab, 10 ul saliva) into a 2 ml collection tube or suitable vessel**, cut off the Samples tiny and as far as possible.

- **approximately 5 mg tissue sample add 95 ul AT1 Buffer and 5 µl AT2 Buffer to the collection tube.**
- **One Swab add 295 ul AT1 Buffer and 5 µl AT2 Buffer to the collection tube.**
- **approximately 10 ul saliva add 85 ul AT1 Buffer and 5 µl AT2 Buffer to the collection tube.**
- **Approximately 3 hair add 45 ul AT1 Buffer and 5 ul AT2 Buffer to the collection tube.**

  Close the tube and vortex briefly. Make sure the sample is covered by the Extraction Solution.

2. Incubate at 56°C for 10 minutes.

3. Incubate at 95°C for 5 minutes.
4. **Add 100 µl AT3 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.