quickAmp™ Tissue Genomic DNA PCR Kit

Storage
The kit should be stored at −20°C.

Procedure
All steps are performed at room temperature unless otherwise noted.

A. Tissue Cell DNA Extraction from Mouse Tails, Animal, Tissues, Hair, or Saliva

1. Pipette 100 µl of Extraction Solution into a microcentrifuge tube or a multiwell plate well. Add 25 µl of Preparation Solution to the tube or well and pipette up and down to mix.

Note: If several extractions will be performed, sufficient volumes of Extraction Solution and Preparation Solution may be mixed in a ratio of 4:1 up to 2 hours before use.

2a. **For Fresh or Frozen Mouse Tails:** Rinse the scissors and forceps in 70% ethanol prior to use and between different samples. Place a 0.5 - 1 cm piece of mouse tail tip (cut end down) into the solution. Mix thoroughly by vortexing or pipetting. Ensure that the mouse tail is in solution.
Note: For fresh mouse tails, perform extractions within 30 minutes of snipping the tail.

2b. **For Animal tissues:** Rinse the scissors or scalpel and forceps in 70% ethanol prior to use and between different samples. Place a 2 – 10 mg piece of tissue into the solution. Mix thoroughly by vortexing or pipetting. Ensure that the tissue is in the solution.

2c. **For Hair Shafts:** Rinse the scissors and forceps in 70% ethanol prior to use and between different samples. Trim excess off of the hair shaft leaving the root and place sample (root end down) into solution. Only one hair shaft, with root, is required per extraction.

2d. **For Saliva:** Pipette 10 µl of saliva into the solution. Mix thoroughly by vortexing or pipetting.

2e. **For Saliva Dried on Card:** Pipette 50 µl of saliva onto collection card and allow the card to dry. Rinse the punch in 70% ethanol prior to use and between different samples. Punch a disk (preferably 1/8 inch or 3 mm) out of the card from the area with the dried saliva sample. Place disk into the solution. Tap tube or plate on hard surface to ensure disk is in solution for incubation period.

3. Incubate samples at room temperature for 10 minutes.

4. Incubate samples at 95°C for 3 minutes.
Note: Tissues will not be completely digested at the end of the incubations. This is normal and will not affect performance.

5. Add 100 µl of Neutralization Solution to sample and mix by vortexing.

6. Store the neutralized tissue extract at 4°C or use immediately in PCR. Continue with Section C, Step 1.

B. Cell DNA Extraction for Buccal Swabs

1. Collect buccal cells on swab and allow the swab to dry for 10 to 15 minutes.

2. Pipette 200 µl of Extraction Solution into a 1.5 ml microcentrifuge tube. Add 50 µl of Preparation Solution to the tube and pipette up and down to mix.

Note: If several extractions will be performed, sufficient volumes of Extraction Solution and Preparation Solution may be mixed in a ratio of 4:1 up to 2 hours before use.

3. Place dried buccal swab into the solution and incubate at room temperature for 1 minute.

4. Twirl swab in solution 10 times and then twirl swab firmly against the side of the tube to remove excess solution into the tube. Discard the swab. Close the tube and vortex briefly.

5. Incubate samples at room temperature for 10 minutes.

6. Incubate samples at 95°C for 3 minutes.

7. Add 200 µl of Neutralization Solution to sample and mix by vortexing.

8. Store the neutralized extract at 4°C or use immediately in PCR. Continue with Section C, Step 1.

C. PCR Amplification

ACTGene *Taq* mastermix, ACTGene Hot-Start *Taq* mastermix, ACTGene Blue/Red *Taq* mastermix, or ACTGene Blue/Red Hot-Start *Taq* mastermix can be used for the following PCR amplification steps. Typical final primer concentrations are approximately 0.4 µM each. Primer concentrations and thermal cycling parameters should be optimized depending on the system.

1. Add 10 µl of PCR mastermix (2x), 4 µl of tissue extract, and proper amount of primers to a thin-walled PCR microcentrifuge tube or plate. Bring the total volume to 20 µl with PCR grade water. Gently mix well.

Note: If less than 4 µl of tissue extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction Solution : Neutralization Solution to compensate the volume of tissue extract to 4 µl.

2. Perform thermal cycling using optimized amplification parameters.

3. Load the amplified DNA onto an agarose gel after the PCR is completed.