Promega

Product Application

Isolation of Genomic DNA from Mouse Tails using the Maxwell® RSC Whole Blood DNA kit on the Maxwell® RSC 48 with automated cartridge preparation using the Maxprep™ Liquid Handler.

Isolate genomic DNA from mouse tail snip using the Maxprep™-Maxwell® automated workflow.

Kit: Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520)

Analyses: UV absorbance, dye-based quantitation, qPCR

Sample Type(s): Mouse tail snips

Input: 0.5cm tails snips (from 4.5mg to 6.2mg)

Materials Required:

- Tissue Lysis Buffer (Cat.# A5091)
- Alkaline Protease, (Cat.# A1721)
- RNase A Solution (Cat.# A7974)
- Thermomixer Eppendorf
- Maxprep™ Liquid Handler (Cat.# AS9201)
- Maxwell® RSC 48 Instrument (Cat.# AS8500)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM455, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Prepare digestion mix : 265µl TLA Buffer, 30µl Alkaline Protease, 5µl RNase A Solution (per reaction). Prepare more digestion mix to include 20µl dead volume per reaction.
2. Cut mouse tail snips (0.5 cm).
3. Cut snips in half and place them into 1.5ml microtubes, add 320µl of digestion mix.
4. Heat at 55°C with vigorous agitation (Thermomixer Eppendorf, 1500rpm) for 30 minutes to 1h (until mouse tail is completely digested).
5. Prepare Maxprep™ Preprocessing as described in section 5 of the technical manual (TM455).
6. Place cartridges in the Maxwell® RSC 48 deck tray(s). Carefully peel back the seals. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).
7. Select Whole Blood Preprocessing on the Maxprep™ software.
8. Prepare the run following step-by-step Maxprep™ software indications: choose 300µl input volume and 150µl elution volume. Load sample tubes containing mouse tails lysates on the tube carrier.
9. Start the Preprocessing run.
10. After cartridge preparation, transfer the Maxwell® RSC deck tray from the Maxprep™ to the Maxwell® RSC 48 Instrument and run the Whole Blood DNA protocol.

Results:

DNA was isolated from four mouse tail snips using the Maxwell® RSC Whole Blood DNA Kit following the above protocol. Performances of this protocol is shown below.

Figure 1: Mouse tail snips digestion. Representative images of the digestion step.

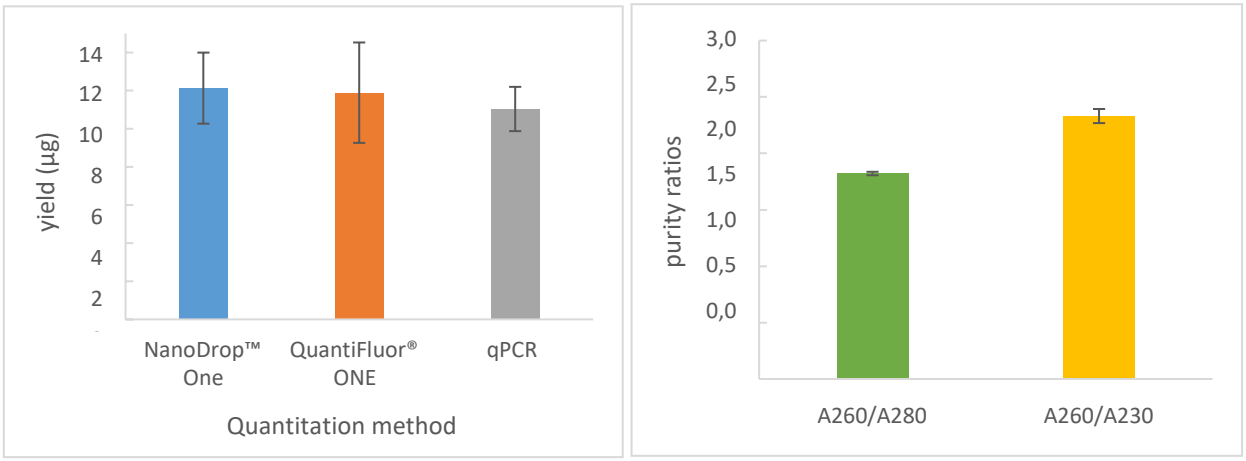

Figure 2: Quantitation of DNA isolated from mouse tail snips using the Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520) on the Maxwell® RSC 48 (Cat.# AS8500) with automated cartridge preparation using the Maxprep™ Liquid Handler (Cat.# AS9201). Left: Purified DNA were quantified using NanoDrop™ One spectrophotometer, using QuantiFluor® ONE dsDNA System (Cat.# E4871) on a Quantus™ Fluorometer (Cat.# E6150) with Lambda DNA standard and by qPCR, using the GoTaq® qPCR Master Mix (Cat.# A6001) on a BioRad CFX96 instrument. Average yields were calculated for 150µl elution volume. qPCR amplification was performed using mouse TERT primers with 93,3% efficiency; R^2= 0,99 and no inhibition observed on non-diluted eluates. **Right**: Purity of DNA isolated. Absorbances were read at 230, 260, and 280nm on a NanoDrop™ One Spectrophotometer and ratios were calculated to access purity. Shown is the AVG ± SD for 4 replicates.