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Auto-Mag® PCR-Pure

Cat # AMD-S002 Version 2.0

Protocol for removing adapter dimers (96-well Plate Format)

This protocol can be used to remove un-ligated adapters and adapter dimers after an adapter addition reaction in NGS library construction.

Materials and Equipment to be supplied by User:

- 100 % ethanol
- Elution Buffer (diH_2O, 10mM Tris-HCl pH 8.0 or TE buffer)
- For 96 well format: 96 well cycling plate
- Appropriate magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipettor and Disposable pipette tips

Before Starting

- Prepare fresh 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).
- Bring the Auto-Mag® PCR-Pure to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure by vortexing.

Procedure

1. Completely float the Auto-Mag® PCR-Pure reagent until it appears homogeneous in color.

2. Add 0.8 volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-100ul adapter addition reaction sample according to the instructions in the table below.

Table 4: Some common adapter addition reaction volumes and suggested Auto-Mag® PCR-Pure Suspension volumes

Adapter addition reaction Sample Volume (ul)	Auto-Mag® PCR-Pure Volume (ul)*
25	20
50	40
65	52
100	80

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for the sample: Auto-Mag® PCR-Pure reagent volume per reaction = 0.8 X sample volume.*

3. Mix thoroughly the Auto-Mag® PCR-Pure reagent and DNA sample by pipetting up and down 10 times. Incubate the mixture for 5 minutes at room temperature.

4. Place the sample plate on the 96 well magnetic separation device and allow Auto-Mag® RNA-Pure beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

5. Keep the sample plate on the magnet and add 200µl of 80% ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second 80% ethanol wash.

7. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

Note: It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.

8. Remove the sample plate from the magnetic separation device. Add 20-50µl of elution buffer (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each well and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

Note: Pre-warming the elution buffer at 55°C can increase the yield.

9. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

10. Transfer the eluate (cleared supernatant) to a new plate for the next step of NGS library construction process.

Note: If necessary, perform a second 0.8x bead-based clean-up.

Additional clean-up

11. Add 0.8 volume of Auto-Mag® PCR-Pure reagent to the supernatant collected from step 10.

12 Mix thoroughly the Auto-Mag® PCR-Pure reagent and sample by pipetting up and down 10 times. Incubate the mixture for 5 minutes at room temperature.

13. Repeat steps 4-7 (capture and washes)

14. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of RNase/DNase free elution buffer, (reagent grade water, Tris-HCl pH 8.0 or TE buffer) to each sample and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

15. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

16. Transfer the eluate (cleared supernatant) to a new plate for the next step of NGS library construction process.