Auto-Mag® NGS Clean-up and Size selection Kit

Cat # AMD-S013 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
- For 96 well format: 96 well cycling plate
- Magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipette and Disposable pipette tips
- 80% ethanol for additional DNA Wash.

Before Starting

- Prepare DNA Wash Buffer according to Preparation of Reagents Section.
- If kit was stored at 2-8°C, bring the Auto-Mag® PCR-Pure reagent to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure reagent 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 10-100µl adapter addition reaction sample according to the instructions in the table below.

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

Adapter addition reaction Sample Volume (µl)	Auto-Mag® PCR-Pure Volume (µl)*
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 sample plate for 5 minutes at room temperature.

4. Place the sample plate on the 96 well magnetic separation device and allow magnetic 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 beads while aspirating the supernatant.

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

Note: DNA Wash Buffer must be diluted with ethanol prior to use. Do not disturb the attracted beads while aspirating the supernatant. Significant bead loss will result in reduced yield.

6. Repeat steps 5 for second DNA wash.

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

Note: It is critical to completely remove all traces of liquid 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 an appropriate volume (20-50µl) of Elution Buffer to each well and mix by pipetting up and down 10 times. Incubate the sample plate 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 sample plate 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 Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate the sample plate 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.