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

Cat # AMD-S013 Version 2.0

Protocol for NGS library clean-up (96-well Plate Format)

This protocol can be used for NGS library clean-up to remove contaminants (such as, nucleotides, primers, adapters, enzymes, buffer additives, salts) and shorter DNA fragments, less than150–200 bp from NGS library samples.

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 1.0 volume of Auto-Mag® PCR-Pure reagent into the reaction plate containing 10-100µl DNA sample according to the instructions in the table below.

Table 3: Some common reaction volumes and suggested Auto-Mag® PCR-Pure Suspension volumes

DNA Sample Volume (µl)	Auto-Mag® PCR-Pure Volume (µl)*
10	10
20	20
25	25
50	50
100	100

** Formula used to calculate the volume of Auto-Mag® PCR-Pure reagent needed for DNA sample: Auto-Mag® PCR-Pure reagent volume per reaction = 1.0 X DNA 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 the 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 to each well and incubate the sample plate 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 a 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 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 an appropriate volume (20-50 µl) of Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate for 5 minutes at room temperature.

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 an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.