PCR Clean up

(Post PCR Clean up System)

Introduction

PCR clean up system is based on paramagnetic bead technology, designed for an efficient purification of amplicons. The purification consists of removal of salts, primers, primer-dimers, dNTPs, as DNA fragments are selectively bound to the magnetic beads particles; and highly purified DNA is eluted with low salt elution buffer or water and can be used directly for downstream applications. GenXion® PCR is designed for either manually or automated on standard liquid handling instruments.

This Clean up can be used for manual procedure as well as guideline for adapting the kit to automatic liquid handling instruments. For the availability of ready-to-run scripts please contact sole distributor directly.

Amplicons purified with GenXion® system are ready to be used in the following applications:

- ✧ PCR
- ✧ Mutation detection and Genotyping
- ✧ Sequencing (Sanger and Next Generation)
- ✧ Microarrays
- ✧ Restriction enzyme clean up
- ✧ Cloning

Process

GenXion® PCR uses a simple 3 steps procedure: Bind-Wash-Elute. GenXion® PCR is added to the PCR reaction sample. The Clean utilizes a magnet separation device for processing the PCR reaction sample. During the process, contaminants and salts are washed off and pure DNA is eluted, ready to be used in subsequent applications.

GenXion® PCR Clean up specifications

Product Number	Description	Number of reactions	Storage condition
GenXion® PCR6005	5 mL	**277**	**2-8° C**
GenXion® PCR6050	50 mL	**2777**	
GenXion® PCR6500	500 mL	**27,780**	

** Number of preps is based on 10µL reaction volume. Volume of GenXion® PCR per reaction= 1.8 x (PCR Reaction Volume)

Materials Supplied in the Kit

GenXion® PCR paramagnetic beads solution

Equipment and Reagents to Be Supplied by User:

- 70% Ethanol
- 10mM TRIS-HCL pH:8.0 (DNA elution)
- Reagent grade water
- 1mM EDTA

Magnet (Stand and Plate):
For 96 well format: 96 well ring stand For 384
well format: 384 Magnet

Reaction Plate:
For 96 well format: 96 Well Cycling Plate For 384
well format:

Clean up in 96 Well Format:

1. Place 96-well PCR plate on bench and measure the volume of the PCR reaction. Determine if transferring the sample to a processing plate is required.

Note: PCR reactions >20 µL will need to be transferred to a processing plate.

2. Add GenXion® PCR according to the PCR reaction (see table below to determine appropriate olume) **Note. Shake the GenXion® PCR to resuspend the beads that may have settled.**

PCR Reaction Volume (µL)	GenXion® PCR Volume at 1.8X (µL)**
10	18
20	36
50	90

** Following formula is used to determine the volume of GenXion® PCR per PCR reaction:
GenXion® PCR per reaction= 1.8 x (PCR Reaction Volume)

3. Pipet up and down to mix well GenXion® PCR and PCR reaction by pipette (6-8 times).

4. Incubate the mixture for 2 minutes at room temperature for maximum recovery.

5. Place the reaction plate on magnetic separation device for 2-3 minutes for separation or wait until all the beads have cleared the solution.

Note: Bead separation is dependent on the quality of the magnetic separation device.

6. With the plate still on the magnet, remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. Remove supernatant from the opposite side of the well.

7. Add 200µL of 70% Ethanol to each well of the reaction plate.

8. Incubate for 1 min at room temperature.

9. Aspirate out the cleared supernatant and discard. Remove as much of the Ethanol as possible.

10. Repeat step 7-9 for a second wash.

11. Leave plate on magnetic separation device for 3-5 min at room temperature with the particles held against the magnet in order to allow the remaining traces of alcohol to evaporate. Do not overdry the beads as this will significantly decrease yield.

12. Remove the reaction plate from the magnetic separation device.

13. Add 30-40µL of elution buffer (Reagent grade water, TRIS-HCl pH 8.0, or TE buffer) to each well of the reaction plate and pipet up and down to mix (10 times).

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

14. Incubate at room temperature for 2-3 minutes.

15. Place the reaction plate onto the magnetic separation device for 2-3 minute or until the magnetic beads clear from the solution.

16. Transfer the eluate to a new plate for storage. Seal with non-permeable sealing film. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

Clean up for the 384 Well Format:

1. Place the 384-well PCR plate on the bench and measure the volume of the PCR reaction. Transfer the sample to a skirted 384-well PCR plate.

2. Add GenXion® PCR according to the PCR reaction (see table below to determine appropriate volume). Note. Shake the GenXion® PCR to resuspend the beads that may have settled.

PCR Reaction Volume (µL)	GenXion® PCR Volume at 1.8X (µL)**
5	9
7	12.6
10	18

** Following formula is used to determine the volume of GenXion® PCR per PCR reaction:
GenXion® PCR per reaction= 1.8 x (PCR Reaction Volume)

3. Pipet up and down to mix well GenXion® PCR and PCR reaction by pipette (6-8 times).

4. Incubate the mixture for 2 minutes at room temperature for maximum recovery.

5. Place the reaction plate on magnetic separation device for 1-2 minute for separation or wait until all the beads have cleared the solution.

Note: Bead separation is dependent on the quality of the magnetic separation device.

6. With the plate still on the magnet, remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. Remove supernatant from the opposite side of the well.

7. Add 30μL of 70% Ethanol to each well of the reaction plate.

8. Incubate for 1 minute at room temperature.

9. Aspirate out the Ethanol and discard. Remove as much of the Ethanol as possible.

10. Repeat step 7-9 for a second wash.

11. Dry the beads by incubating the separation plate 3-5 min at room temperature with the particles held against the magnet in order to allow the remaining traces of alcohol to evaporate. Do not over dry the beads as this will significantly decrease yield.

12. Remove the reaction plate from the magnetic separation device.

13. Add 30μL of elution buffer (Reagent grade water, TRIS-HCl pH 8.0, or TE buffer) to each well of the reaction plate and pipet up and down to mix (5 times).

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

14. Incubate at room temperature for 2-3 minutes.

15. Place the reaction plate onto the magnetic separation device for 1-2 minute or until the magnetic beads clear from the solution.

16. Transfer the eluate to a new plate for storage. Seal with non-permeable sealing film. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

Size-selection Protocol:

1. Preparation Step:

a) Determine the amount of GenXion® Mag PCR Clean-up solution needed for desired selection range.

Please refer to the table below for general guideline:

Addition Ratio	Selection Range	Peak value (bp)
0.9x~1.1x	200~260 bp	230
0.8x~1.0x	250~320 bp	280
0.7x~0.9x	270~350 bp	300
0.6x~0.8x	300~500 bp	350
0.5x~0.7x	330~700 bp	450
0.4x~0.6x	500~1000 bp	800

The addition ratio designates the volume of reagent by:

PCR Clean-up solution = _____ (addition ratio) × Initial DNA sample volume

b) Gently shake the reagent bottle to fully re-suspend the settled beads

c) Prepare at least 20 µL of elution buffer and 400 µL of 70%-80% ethanol solution (dissolved in bio-molecular grade water) for each reaction.

2. First Binding Step:

a) Add the pre-determined amount of GenXion® Mag PCR Clean-up solution to the DNA sample in a micro-centrifuge tube or 96-plate well. Pipette mix the solution 5 times and incubate at room temperature for 5 minutes. This process binds the larger DNA fragments to the beads and the smaller ones will remain in the solution.

Pulse-spin the solution to collect all the droplets.

*Note: Do not vortex. It is associated with poor binding ratio.

Incubation at 50 °C is optional but not necessary

b) Place the sample on an Macro IMAGTM or other magnetic device to separate the paramagnetic beads from the solution. Wait for 1 min or until the solution completely clears.

c) Transfer the supernatant into a fresh tube, the desired DNA fragments are in the solution.

*Caution: do not transfer the beads along with the cleared supernatant. Incomplete elimination of beads in the first step results in selection variability.

3. Second Binding Step:

a) Add more PCR clean-up mix to the new tube and gently pipette-mix five times.

Amount added = (_____(final ratio) -_____(initial ratio)) × Initial DNA sample volume

b) Pulse-spin the tube to collect all droplets, then incubate at room temperature for 5 minutes. The solution should appear homogenous.

c) Place the tube back on to the magnetic device and wait until the solution clears.

d) While the tube is still on the magnet, aspirate off the supernatant gently. Be extra cautious not to disturb the beads settled on the side of the tube. Loss of beads at this point is associated with poor recovery rate.

*Note: The selected DNA fragments are on the beads.

4. Wash Step:

- **While the reaction plate/tube is still on magnet dispense 200 µL of 70%-80% ethanol to each well. Incubate for 30 seconds at room temperature, gently pipette- mix five times, then aspirate off the ethanol. Repeat for a total of two washes.**

*Elimination of the settle beads will lower the recovery rate of the final product. After the wash step, remove as much of the ethanol as possible because the residual ethanol may interfere with downstream applications.

NOTE: Allowing the plate to stand for 5 minutes at room temperature will enable the remaining ethanol to evaporate completely. Be careful not to dry out the beads completely (the layer of settled beads will appear cracked if this happens). This will reduce elution efficiency and lower the recovery of DNA fragments.

5. Elution Step:

a) Once ethanol is fully eliminated, remove the reaction plate from the magnetic plate and add 20 µL of elution buffer (Reagent grade water, TRIS- HCl pH 8.0, or 10 mM Tris-HCl pH 8.0, 1 mM EDTA) to each well. If the beads remain settled, gently pipette-mix or use a larger elution volume.

The DNA is rapidly eluted off the magnetic beads and it is not necessary for the beads to go back into solution for elution to occur. More than 20 µL of elution buffer can be used if a larger end volume is required, but the final product will be more dilute. Less than 40 µL will require extra mixing to ensure the liquid comes into contact with the beads.

b) To separate the paramagnetic beads from the supernatant place the reaction plate onto a 96 well magnetic plate or IMAG™ for one minute or until the solution becomes clear. The supernatant now contains the eluted DNA. The beads may be discarded.

c) Transfer the supernatant to a fresh plate for storage and downstream processes.

SUPPLEMENTARY PROTOCOL

Recovery of DNA smaller than 125 bp

For optimal recovery of DNA products smaller than 125 bp either add:

i) Isopropanol to the GenXion@ Clean-up reagent prior to use.

-or-

ii) Isopropanol to the bead-sample mix prior to applying the magnet.

1. Add isopropanol to beads prior to starting the purification process.

Mix 70 µL of 100% Isopropanol with 180 µL of Macro Mag PCR Clean-up bead solution to yield a final concentration of 28% isopropanol.

For every 10 µL of PCR product, add 25 µL of bead-isopropanol mix (1:2.5 ratio (product:beads)).

Note. If using automation with the diluted Macro Mag PCR Clean-up reagent, it may be easier to set the PCR sample volume on the robot to 1.4 fold of the actual volume. For instance, if there is 10 µL of the PCR product; add 14 µL into the system so that 25 µL of the diluted Macro Mag PCR reagent will be added instead of the typical 18 µL.

2. Add 100 % isopropanol to the sample-bead mix to yield a final concentration of 20%. For example, add 7 µL of 100 % isopropanol to 28 µL of bead/sample mix (18 µL beads, 10 µL sample).

Removal of primer dimer or DNA smaller than 200 bp

If longer primer sets are to be used and primer dimer contamination occurs, it is possible to remove the primer dimers by using diluted Macro Mag PCR Clean-up reagents.

1. Perform a sequential titration to determine the best dilution of the Macro Mag PCR clean-up reagent for the PCR product.

a) Add 2 µL, 4 µL, or 8 µL of water into 18 µL of Macro Mag PCR Clean-up reagent and test for primer dimer or DNA removal.

b) Use the optimized dilution for subsequent PCR purification.