



MAGNETIC BEADS PCR CLEAN-UP SYSTEM (HighPrep™ PCR, MAGBIO)

Materials

- Magnetic beads HighPrep™ PCR reagent (*Fridge 1*)
- Magnetic stand (for bead separation)
- Ethanol 80%
- Elution buffer (e.g. 10 mM Tris-HCl)
- 20-200 µl pipette tips. At least 2 tips per sample.

Before you begin

Bring the HighPrep™ PCR reagent (magnetic beads) to room temperature for at least 30 min before use.

Protocol

1. Shake thoroughly to fully resuspend the magnetic beads.
2. Add 1.8 µl of resuspended magnetic beads per 1 µl of PCR reaction. Mix by pipetting up and down 6-8 times.

PCR Reaction Volume (µL)	HighPrep™ PCR Volume at 1.8X (uL)*
10	18
20	36
50	90

* Formula used to calculate the volume of HighPrep™ PCR reagent needed for PCR reaction:
HighPrep™ PCR reagent volume per reaction = 1.8 X PCR reaction volume.

Your PCR reaction volume	HighPrep™ PCR reagent to add

3. Incubate the mixture for 5 minutes at room temperature (Figure 1).
4. Place the sample plate on the 96-well magnetic separation rack for 3 minutes or until the solution clears. Beads will stick to the side of the well towards the magnet (Figure 2).
5. With the sample plate still on the magnet, remove and discard the supernatant by pipetting. Make sure not to disturb the beads while aspirating the supernatant.
6. With the sample plate still on the magnet, add 150-200 µL of 80% ethanol to each well and incubate for 30 seconds at room temperature.

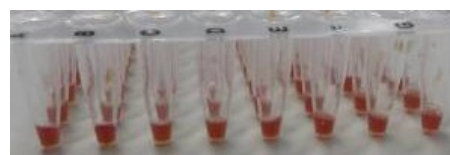


Figure 1: mixed beads



Figure 2: cleared beads



A tip about tips: *If you are careful, dispense the ethanol above the samples so you can reuse the tips.*

7. With the sample plate still on the magnet, remove and discard the supernatant by pipetting.
8. Repeat steps 7-8 for a total of two 80% ethanol washes.
Try to remove as much residual ethanol as possible by pipetting.
9. Dry the beads by incubating the plate for 10-15 minutes at room temperature with the plate still on the magnetic separation device.
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
10. Remove the sample plate from the magnetic separation rack. Add 40 μ L of elution buffer (reagent grade water, 10 mM Tris-HCl, etc.) to each well and pipet up and down 5 times to mix (Figure 3). *Using less elution buffer will not result in a more concentrated product.*
11. Incubate for 2 minutes at room temperature.
12. Place the sample plate back on the magnetic separation rack and wait 1 minute or until the magnetic beads clear from the solution.
13. The DNA is now in solution. The samples can be stored with beads at 4C. Just place them quickly on the magnet before using.

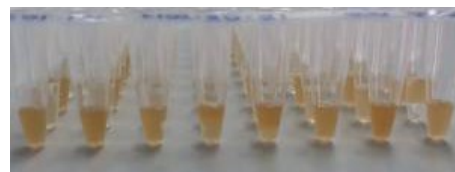


Figure 3: resuspended beads

A note about tip usage

This protocol can burn through a lot of tips, especially in 96-well format. You can run out. Plan accordingly. Typically we would use this protocol for purifying PCR products prior to Sanger sequencing. If this is the case—there will be no further amplifications from your samples—it is possible to re-use tips at some steps. Mixing beads and sample at step 2? This always needs a fresh tip. Steps 6-8 can be done with a single set of tips on the multichannel pipette. Reusing tips as described takes this protocol from 5 tips per sample to 2 tips per sample. **However**, this will cross contaminate samples. Not a problem for pretty much any Sanger sequencing option, you will never see it. But if these samples are to be used in downstream PCR amplification steps, be sure to use fresh tips whenever going into your samples.