GRISP
RESEARCH SOLUTIONS

Xpert DNA Purification Magnetic Beads

#GK39.0025 (25 ml) | GK39.0060 (60 ml) | GK39s (trial size)

(FOR RESEARCH ONLY)

Product: Xpert DNA Purification Magnetic Beads consists of paramagnetic particles coated with carboxyl groups that reversibly bind DNA (Solid Phase Reversible Immobilization). The magnetic beads are supplied in a buffer that has been optimized in order to selectively and reversibly bind DNA fragments from 100bp up to 1kb and even much larger. This allows for easy PCR clean-up, as primers, primer-dimers, dNTPs, enzymes, excess salts, and other impurities can be removed quickly and efficiently by a simple washing procedure. Because this purification method does not require centrifugation or vacuum filtration, it can be readily adapted to 96-well or 384-well microplate automation platforms. Moreover, these beads can be seamlessly integrated into NGS Library preparation workflows.

Applications: PCR clean-up, Size-selection, Cloning, Fragment Analysis, Genotyping, High-throughput, NGS Library preparation, and other downstream applications requiring highly purified DNA.

Content: #GK39.0025 contains 25 ml of Xpert Magnetic Beads, which is sufficient for almost 700 samples of 20µl (standard protocol PCR Clean-up using ratio of beads:sample ratio of 1.8). #GK39.0060 contains 60ml of Xpert Magnetic Beads and #GK39s is a trial sample of1ml.

Component	GK39.0025	GK39.0060	GK39s
Xpert Magnetic Beads	25 ml	60 ml	1 ml

Additional required materials or reagents (not included): 70% ethanol, nuclease-free water, 10mM Tris-Acetate pH 8.0 or TE Buffer and a magnetic separation rack (for tubes or 96-well plates).

Storage: Store tightly sealed at 2 to 8ºC, protected from light, for up to 2 years. DO NOT freeze.

Prior to use

Ensure that Xpert DNA Purification Magnetic Beads have been warmed up to room temperature (~30 min). Prepare sufficient 70% ethanol for the washing steps. Note that 70% ethanol is hygroscopic and should therefore be prepared freshly. Please mix 70ml of 100% ethanol with 30ml of water (resulting in approximately 95ml of 70% ethanol) instead of adding water to 70ml of 100% ethanol until 100ml, as this would lead to 100ml of approximately 65% ethanol as these solvents are miscible in each other.

Immediately before use, resuspend any beads that may have settled by shaking the bottle vigorously. In case of processing large amounts of samples, repeat shaking regularly.

Basic Protocol

This basic protocol for PCR purification using either 96-well or 384-well microplates is based on a beads-to-DNA ratio of 1.8 (e.g., 90µl of beads per 50µl of sample), however, it can easily be adapted for different sample volumes and/or other ratios for size selection. For samples larger than 100µl, it is recommended to split the sample into more wells or use a 1.5-ml microcentrifuge tube with a corresponding magnetic separation rack.

DNA Purification

1. Add Xpert Magnetic Beads to the DNA samples according to the tables below. For other volumes, simply modify proportionally using a beads-to-DNA ratio of 1.8

96-well microplate			384-well microplate	
sample (µl)	beads (µl)		sample (µl)	beads (µl)
5	9		2	3.6
10	18		5	9
20	36		7	12.6
50	90		10	18
100	180		12.5	22.5

2. Mix thoroughly to a homogenous appearance by pipetting up and down the entire mixture 10 times. Allow for optimal DNA binding by incubation at room temperature for 5 minutes.
3. In order to separate the magnetic beads from the solution, place the microplate onto a magnetic separation rack for 5 minutes (Ensure the solution has becomes clear before proceeding to step 4).

   During steps 4 to 8 maintain the microplate on the magnetic separation rack at all times.
4. Carefully aspirate off the cleared solution and discard. Avoid disturbing the magnetic beads.
5. Dispense 70% ethanol to each well: 200µl/well in case of a 96-well plate or 30µl/well in case of a 384-well plate.
6. Incubate at room temperature for 30 seconds.
7. Carefully aspirate off the ethanol and discard. Avoid disturbing the magnetic beads.
8. Repeat steps 5-7 once, then allow the plate to air-dry for 3-5 minutes to remove any residual ethanol.

9. Remove the microplate from the magnetic rack and elute DNA with 10-50µl/well (as desired) of elution buffer (e.g., nuclease-free water, TE, 10mM Tris-HCl pH 8.0 or 10mM Tris-acetate pH 8.0).
10. Mix thoroughly by pipetting up and down the entire mixture 10 times.
11. Incubate at room temperature for 2-5 minutes.
12. Place the microplate back onto the magnetic separation rack for 5 minutes.
13. Carefully transfer the eluent to a new plate. In case of carryover of beads, repeat step 12 and transfer into another new plate (or tube).
14. Store purified DNA at -20°C or proceed with downstream application.

Size Selection

The basic protocol is optimized for PCR Clean-up. However, the protocol can be easily adapted for other applications by using different beads:DNA volume ratios. In general, the higher the ratio the smaller the fragments that are captured. For example, using a ratio of 0.8x instead of 1.8x would results in the removal of DNA fragments smaller than 250bp (instead of smaller than 100bp), or alternatively the removal of DNA fragments that are larger than 250bp if the unbound fraction was retained.

Table 1. Recommended Beads-to-DNA ratio for Size Selection

Component	150bp	200bp	250bp	300bp	400bp	500bp	600bp	700bp
Beads-to-DNA ratio	1.50	0.90	0.80	0.70	0.60	0.55	0.50	0.45

In the table above, one can find the recommended beads-to-DNA ratio for the purification of DNA above a desired minimum size. For example, when starting with a 50µl sample and using 45µl of Xpert DNA Purification Magnetic Beads (ratio of 0.90), the final product would be purified DNA of size of 200bp and larger, whereas if one would use 40µl of beads (ratio of 0.80), the final product would not include 200bp sized DNA but only DNA of 250bp and larger. And so on. So, one just needs to adapt step 1 of the basic protocol to the desired beads-to-DNA ratio. It is highly recommended to use samples of at least 50µl, as lower volumes will decrease pipetting accuracy and hence increase selection point variability.