sbeadex PCR clean-up kit

Catalogue number **41701**
(For research use only. Not for use in diagnostic procedures.)

Description

sbeadex™ kits use magnetic separation for the purification of nucleic acids. Superparamagnetic particles coated with sbeadex surface chemistry are used to capture nucleic acids under defined buffer conditions. The three simple steps of the sbeadex protocol means that more time can be spent on downstream processes. Furthermore, magnetic microparticles are ideally suited for high throughput laboratories which utilise automated liquid handling systems or multi-pipetting units (8, 12 or 96 channel pipettes).

Kit uses

sbeadex PCR clean-up kits are used to purify PCR products. Components of the PCR reaction such that primers, unincorporated nucleotides, enzymes and other contaminations are efficiently removed during the purification process due to the chemical specificity and physical size of sbeadex particles. The method was developed and optimised using 20 µL of PCR product from standard PCR mixtures which were subsequently analysed by Sanger sequencing. The protocol can be scaled up or down for other volumes of PCR product, however care should be taken to maintain the buffer volume ratios specified in the protocol.

For information on protocols for other starting materials please contact our application specialists via email: extraction@lgcgenomics.com or Tel: +49 (0)30 5304 2250.

Kit content

	Colour	Cat. **41701**
Binding buffer PCR	Green	5.5 mL
sbeadex particle suspension	White	220 µL
Elution buffer AMP	Black	3 mL

Additional required reagents:

- 70% ethanol

Additional buffers can be purchased separately, catalogue numbers available on request.

Storage

Kit components should be used within six months of delivery and stored under the recommended conditions. Please refer to the kit box label for the expiry date.

Room temperature
Binding buffer PCR
sbeadex particle suspension
Elution buffer AMP

Reagent preparation

sbeadex particle suspension

Mix the suspension thoroughly before use to fully re-suspend the particles.

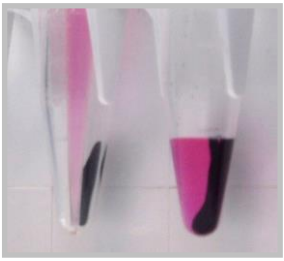
Manual protocol (purification of 20 µL reaction mixture)

1. Ensure the **sbeadex particles** are fully re-suspended
2. Add 50 µL of **Binding buffer PCR** and 2 µL of **sbeadex particles** to each sample. Mix thoroughly, set pipette volume to 50 µL and pipette up and down 10 times
3. Incubate for 5 minutes at room temperature to allow sufficient time for binding to occur. Tip mix for 30 seconds after 1.5 minutes and 3.5 minutes of incubation time has elapsed
4. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the sbeadex particles to form a pellet
5. Remove the supernatant and discard. Ensure as much of the supernatant is removed as is possible without dislodging the particle pellet
6. Move the magnet away from the sample tubes
7. Add 150 µL of **70 % ethanol** and re-suspend the pellet. Mix thoroughly, set pipette volume to 100 µL and pipette up and down 5 times or until pellet is fully re-suspended
8. Incubate at room temperature for 5 minutes, agitating the sample during the time period. Use a shaker or vortex periodically
9. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the sbeadex particles to form a pellet
10. Remove the supernatant and discard. Ensure as much of the supernatant is removed as is possible without dislodging the particle pellet
11. Move the magnet away from the sample tubes
12. Add 20 µL of **Elution buffer AMP** and re-suspend the pellet. Mix thoroughly, set pipette volume to 10 µL and pipette up and down 20 times or until pellet is fully re-suspended
13. Incubate at room temperature for 5 minutes, tip mix 20 times after 1.5 minutes and 3.5 minutes of incubation time has elapsed
14. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the sbeadex particles to form a pellet
15. Remove the eluate and place into a new sample tube. To avoid particle transfer it is recommended to transfer only 15 µL of the eluate.

Note: The sbeadex PCR clean-up kit has been optimised to purify PCR product from 20 µL PCR reaction mixture. The protocol can be scaled up or down for other volumes of PCR mixture volumes, however care should be taken to maintain the buffer volume ratios specified in the protocol

Tips for manual protocol

For manual testing of the protocol or if no magnet is available it is recommended to spin tubes for 10 seconds to enable the magnetic particles to form a pellet.



When removing supernatants it is important to remove as much of the liquid as possible without dislodging the particle pellet. With magnets used for manual protocols the particle pellet forms on the back wall of the sample tube. When placing the pipette tip inside the tube be sure to aim the end of the tip to the front wall of the sample tube to avoid disrupting the particle pellet.

To remove as much liquid as possible it is recommended to aspirate once, let any liquid run down the walls of the tube and then aspirate a second time to remove these remnants of liquid.

Tips for automated protocol

Follow the manual protocol as specified overleaf in respect to volumes. Tips on automated mixing are given below:

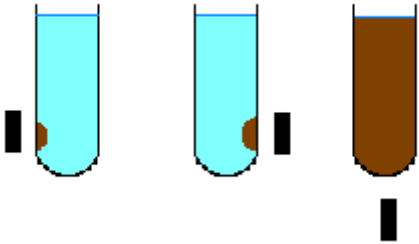
Mixing with automated liquid handling system

- Set mixing volume to be between 50 % to 80 % of the volume to be mixed (instrument dependent)
- For each mixing step aspirate and dispense between 5 and 10 times depending on the efficiency of the liquid handler
- Increase aspirate and dispense speeds when re-suspending pellets to ensure complete re-suspension.

Tips for automated protocol

Using sep™ boxes

- sep boxes are computer driven magnetic particle collectors with active cooling and heating functionality
- Depending on the sep box used the volumes specified in the manual protocol may need to be changed to be within their maximum working volume. Note: sep 96 x *0.2* has a maximum working volume of 180 µL.
- The magnets can be placed in three positions in relation to the sample – left, right and underneath (away from the sample)



- For effective re-suspension of particle pellets it is recommended to move the magnets from the left to right positions using the ‘cycle mode’. See sep box operating manual for more details
- For efficient elution of the nucleic acids from the particles it is recommended to use the ‘cycle mode’ during the elution incubation period.

Troubleshooting

Problem	Possible cause	Corrective action
Low yield	Inefficient binding	Ensure that the Binding buffer PCR and sbeadex particles are mixed thoroughly
Particles present in eluates	Aspirating too fast	Reduce the speed at which supernatants are removed
	Loose pellet	Increase separation time to allow time for a tighter pellet to form
	Disrupting pellet during aspiration	Position tip further away from pellet whilst removing supernatants

Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure
- Binding buffer PCR contains high concentrations of detergent and salt. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Binding buffer PCR contains up to 50 % n-propanol. Keep away from naked flames.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Binding buffer PCR	 Danger	H225/H302/H315/ H318/H336	P210/P303+P361+P353/P305 +P351+P338/P310/P405
sbeadex particle suspension	-	-	-
Elution buffer AMP	-	-	-

SDS (Safety data sheet) are available at our “Genomics Resource Center” on our webpage www.lgcgroup.com/genomics.

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