Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure
- Binding buffer BL and mag particle suspension BL contain high concentrations of salts and detergents. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Note Binding buffer BL can yellow over time, this change of colour does not affect the performance of the buffer
- Prepared Wash buffer BL 1 and Wash buffer BL 2 contain up to 70 % organic solvents. Keep away from naked flames.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer P	 Warning	H319/H400	P264/P273/P280/P305+P351+P338/ P337+P313/P501
Binding buffer BL	 Danger	H302+H312+H332 /H314/H412	P260/P280/P305+P351+P338/ P312/P405/P501
mag particle-suspension BL	 Danger	H314	P260/P303+P361+P353/P305+P351+ P338/P310/P405/P501
Wash buffer BL 1 (concentrate)	-	-	-
Wash buffer BL 2 (concentrate)	-	-	-
Elution buffer BL	-	-	-

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

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40610/4.00-2016-06

mag plant kit

Catalogue number **40601** and **40610**
(For research use only. Not for use in diagnostic procedures.)

Description

mag™ kits use magnetic separation for the preparation of nucleic acids. Superparamagnetic particles coated with mag surface chemistry are used to capture nucleic acids from a sample. The nucleic acid/particle complex is subsequently washed to remove impurities. The nucleic acid is then eluted from the particles and ready for use in downstream processes.

Kit uses

mag plant kits are used to extract DNA from a wide variety of plant types. The method was developed and optimised using plant leaves with an average diameter of 9 mm (about 5-10 mg lyophilised or 10-30 mg of fresh leaf material) from the following plants:

- Tomato
- Pepper
- Lettuce
- Maize
- Sunflower
- Rapeseed

For information on protocols for other starting materials please contact our application specialists via email: info.de@lgcgroup.com or Tel: +49 (0)30 5304 2200.

Kit content

	Colour	Cat. **40601**	Cat. **40610**
Lysis buffer P	Blue	15 mL	100 mL
Binding buffer BL	Green	15 mL	200 mL
mag particle suspension BL	White	1.2 mL	11 mL
Wash buffer BL 1 (concentrate)	Red	8,6 mL	57 mL
Wash buffer BL 2 (concentrate)	Yellow	4.5 mL	30 mL
Elution buffer BL	Black	15 mL	100 mL

Additional required reagents:

- Ultra pure sterile water
- Ethanol
- Acetone
- Isopropanol
- Mercaptoethanol or 1- Thioglycerol (see note on polyphenolic compounds in 'Reagent preparation' section)

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

Storage

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

Room temperature
Lysis buffer P
Binding buffer BL
mag particle suspension BL
Wash buffer BL 1
Wash buffer BL 2
Elution buffer BL

Troubleshooting

Problem	Possible cause	Corrective action
PCR inhibition	Incomplete buffer removal	Ensure all the buffer is removed before adding the next buffer. Check and if necessary adjust the liquid handling parameters for automated systems
Low yield	Inefficient binding	Ensure that the lysate, Binding buffer BL and mag particles are mixed thoroughly
	Wash buffer BL 2 acetone composition <70 %	Ensure that the Wash buffer BL 2 bottle is closed tightly when not in use to prevent acetone evaporation
Coloured eluates	Incomplete buffer removal	Ensure all the buffer is removed before adding the next buffer. Check and if necessary adjust the liquid handling parameters for automated systems
	Heavily stained sample material	Contact our technical specialists for advice
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
Low ratio between A_{260} and A_{280}	Acetone carryover in eluate	Acetone has a maximum UV absorbance at 268 nm and a A_{260}/A_{280} of 1.53. If this phenomenon occurs prolong the drying time to ensure all the acetone evaporates

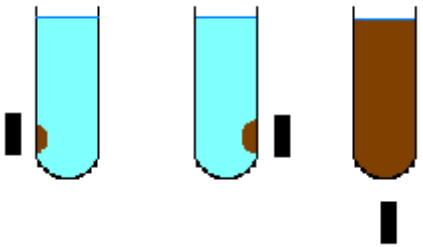
Tips for automated protocol

Mixing with automated liquid handling system (cont'd.)

- Keep mix aspirate and dispense speeds low with Lysis buffer P and Binding buffer BL to avoid frothing
- Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete re-suspension.

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.

Reagent preparation

Presence of precipitates

Salt precipitates can form in Lysis buffer P, Binding buffer BL and mag particle suspension BL at low temperatures. Check for the presence of precipitates prior to use and if required re-dissolve them by incubating the reagents at 37 °C.

Use of Lysis buffer P with polyphenolic compounds

For plant material which contains polyphenolic compounds it is recommended that 1 % mercaptoethanol or 1 % 1-thioglycerol be added to Lysis buffer P. This spiked solution must be used immediately after preparation.

mag particle suspension BL

The mag particles are suspended in a specially formulated buffer which avoids rapid sedimentation or clogging of particles during handling. Mix the suspension thoroughly before use to fully re-suspend the particles.

Wash buffer BL 1

Prepare the Wash buffer BL 1 according to the instructions on the bottle label. For kit catalogue number 40601 add 7 mL of ethanol and 7 mL of isopropanol. For kit catalogue number 40610 add 63 mL of ethanol and 60 mL of isopropanol. Ensure the lid is closed tightly when the bottle is not in use to avoid evaporation.

Wash buffer BL 2

Prepare the Wash buffer BL 2 according to the instructions on the bottle label. For kit catalogue number 40601 add 7 mL of acetone. For kit catalogue number 40610 add 70 mL of acetone. Ensure the lid is closed tightly when the bottle is not in use to avoid evaporation.
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Manual protocol

1. Add 90 µL of **Lysis buffer P** to each dry grinded sample and mix (see notes on options for disruption and homogenization of plant material)
2. Incubate at 65 °C for at least 10 minutes then centrifuge at 2 500 g for 10 minutes
3. Add 110 µL of **Binding buffer BL** to a fresh sample tube
4. Ensure the **mag particle suspension BL** is fully re-suspended. Add 10 µL to the tubes containing the binding buffer
5. Transfer 45 µL of **lysate** to the tubes containing the binding buffer/mag particles. Mix thoroughly, set pipette volume to 150 µL and pipette up and down 5 times
6. Incubate for 2 minutes at room temperature to allow sufficient time for binding to occur
7. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the mag particles to form a pellet
8. Remove the supernatant and discard. Ensure as much of the supernatant is removed as possible without dislodging the particle pellet
9. Move the magnet away from the sample tubes
10. Add 130 µL of **Wash buffer BL 1** 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
11. Incubate at room temperature for 10 minutes, agitating the sample during the time period. Use a shaker or vortex periodically
12. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the mag particles to form a pellet
13. Remove the supernatant and discard. Ensure as much of the supernatant is removed as possible without dislodging the particle pellet
14. Repeat steps 9 to 13 with 70 µL of **Wash buffer BL 2**
15. Dry the pellet at 55 °C for 5 minutes. Sample tubes must be left open to allow evaporation to occur
16. Add 63 µL of **Elution buffer BL** and re-suspend the pellet. Mix thoroughly, set pipette volume to 50 µL and pipette up and down 5 times or until pellet is fully re-suspended
17. Incubate at 55 °C for 10 minutes, agitating the sample during the time period. Use a heated shaker or vortex periodically
18. Bring magnet into contact with the sample tubes. Wait for 3 minutes at room temperature to allow the mag particles to form a pellet
19. Remove the eluate and place into a new sample tube. To avoid particle transfer it is recommended to transfer only 50 µL of the eluate.

Disruption and homogenisation of plant material

To get high molecular weight DNA it is recommended to lyophilise the plant material prior to homogenisation. Please grind dry plant material without addition of Lysis buffer.
Fresh (not dried) plant material should be homogenised after addition of 90 µL Lysis buffer P or can be placed in liquid nitrogen prior grinding.

When processing small numbers of samples grind material:

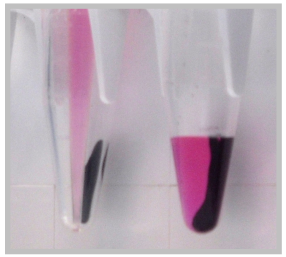
- With a mortar and pestle with sea sand
- In a 1.5 mL sample tube using a micro pestle
- In a 1.5 mL/ 2 mL sample tube using a steel ball and a ball mill

When processing large numbers of samples grind material:

- In a rack of sample tubes i.e. 96 array of 1.4 mL tubes using steel balls and a ball mill
- In a 96 well sealed plate using steel balls and a ball mill

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