Kleargene plant spin plate DNA extraction kits user manual

Contents of this guide

1. Product description and specification

The Kleargene family are a series of kits designed to enable simple, rapid extraction and purification of genomic DNA from a variety of sources. The Kleargene plant plate DNA kit is designed for the extraction of DNA from small amounts (20 - 30 mgs) of plant material. The method is based on the highly proven technology of detergent-driven cell lysis, followed by guanidinium isothiocyanate-mediated DNA binding to glass fibre membranes encapsulated in a filter plate. Contaminants are removed by washing and DNA is subsequently eluted into a low salt buffer. The entire process is carried out in one well per sample, and full 96-well or 384-well plates of samples can be extracted in under an hour.

2. Kit contents

Component	Storage[1]	KBS-1012-201	KBS-1012-202	KBS-1012-210	KBS-1012-211
Buffer L1	20-25ºC	15 mL	60 mL	250 mL	1000 mL
Buffer C1	20-25ºC[2]	30 mL	125 mL	500 mL	2 x 1000 mL
Buffer A1	20-25ºC	15 mL	60 mL	250 mL	2 x 500 mL
Buffer W1	20-25ºC	4.5 mL	18 mL	75 mL	300 mL
Buffer E1	20-25ºC	15 mL	60 mL	250 mL	1000 mL
Filter plate	20-25ºC	1 x 96	4 x 96	16 x 96	64 x 96

Table 1: Contents of Kleargene 96-well plant kits

Component	Storage[1]	KBS-1012-204	KBS-1012-205	KBS-1012-212	KBS-1012-213	KBS-1012-214
Buffer L1	20-25ºC	60 mL	250 mL	1000 mL	5000 mL	25000 mL
Buffer C1	20-25ºC[2]	125 mL	500 mL	2 x 1000 mL	2 x 5000 mL	2 x 25000 mL
Buffer A1	20-25ºC	60 mL	250 mL	2 x 500 mL	2 x 2500 mL	2 x 12500 mL
Buffer W1	20-25ºC	18 mL	75 mL	300 mL	1500 mL	7500 mL
Buffer E1	20-25ºC	60 mL	250 mL	1000 mL	5000 mL	25000 mL
Filter plate	20-25ºC	1 x 384	4 x 384	16 x 384	80 x 384	400 x 384

Table 2: Contents of Kleargene 384-well plant kits

[1] – before the required additional reagents are added to each buffer

[2] – this should be stored in the dark

To be supplied by the user

Additional reagents

- Ethanol
- Isopropanol (propan-2-ol)
- β-mercaptoethanol (2-mercaptoethanol)
- α-amylase (**ONLY for seed material**, Sigma-Aldrich cat. no. A3403 recommended).

Equipment needed

- Tissue homogenisation instruments and consumables
- Manual pipettes and disposable pipette tips OR a microplate dispensing robot
- Polypropylene storage plates (and optionally 'reservoir' plates)
- Fan oven
- Waterbath incubator
- Personal protective equipment (lab coat, gloves, safety glasses).

3. Reagent preparation

- Precipitates can form in the lysis and binding buffers after prolonged low-temperature storage (incubate at 37°C if this is the case and mix well until clear).
- Ensure that the buffers are well mixed before use, following the addition of the necessary reagents. (see Tables 3 and 4 below)

Component	Reagent to add	1 x 96	4 x 96	16 x 96	64 x 96
Buffer L1	β-mercaptoethanol	120 µL	480 µL	2 mL	8 mL
Buffer C1	β-mercaptoethanol	240 µL	1 mL	4 mL	2 x 8 mL
Buffer A1	Isopropanol	7.5 mL	30 mL	125 mL	2 x 250 mL
	Ethanol	7.5 mL	30 mL	125 mL	2 x 250 mL
Buffer W1	Ethanol	10.5 mL	42 mL	175 mL	700 mL

Table 3: Reagents to be added to buffers from 96-well plate kits

Component	Reagent to add	1 x 384	4 x 384	16 x 384	80 x 384	400 x 384
Buffer L1	β-mercaptoethanol	480 µL	2 mL	8 mL	40mL	200 mL
Buffer C1	β-mercaptoethanol	1 mL	4 mL	2 x 8 mL	2 x 40mL	2 x 200 mL
Buffer A1	Isopropanol	30 mL	125 mL	2 x 250 mL	2 x 1250mL	2 x 6250 mL
	Ethanol	30 mL	125 mL	2 x 250 mL	2 x 1250mL	2 x 6250 mL
Buffer W1	Ethanol	42 mL	175 mL	700 mL	3500 mL	17,500 mL

Table 4: Reagents to be added to buffers from 384-well plate kits

- The volumes of each reagent to add are also declared on the labels of the bottles in the 4 x, 16 x, 64 x, 80 x and 400 x kits; space constraints prevent this on the bottles of the 1 x kit.

Buffers L1 & C1

These buffers require the addition of 100% β-mercaptoethanol before use. It is recommended that any buffer to which β-mercaptoethanol is added should be used immediately. L1 and C1 should be aliquoted appropriately if not to be used in its entirety. The enzyme α-amylase may also be added to L1 if seeds are being extracted (see the 'Notes' section at the end of this document for information regarding its use). If required, it is recommended that α-amylase is added to L1 to a final concentration of 300U / mL.

Buffers A1 & W1

Buffer A1 requires the addition of ethanol and isopropanol. Buffer W1 requires the addition of ethanol only. It is not necessary to aliquot these buffers before use. It is, however, recommended that any unused buffer containing these alcohols should be stored out of direct sunlight, and that the bottle caps are securely tightened to ensure that no evaporation occurs.

4. Safety information

DO NOT ADD BLEACH OR ACIDIC SOLUTIONS DIRECTLY TO THE SAMPLE PREPARATION WASTE.

- The sample preparation waste contains guanidinium isothiocyanate which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.
- It is highly recommended that personal protective equipment is worn throughout the extraction process.
- For more detailed information, please refer to the safety data sheets (SDS).

5. Protocol

(also available in outline form on our website)

THE VOLUMES OF EACH BUFFER USED IN THE PROTOCOL ARE SCALABLE IN PROPORTION TO THE AMOUNT OF STARTING MATERIAL EXTRACTED; THE VOLUMES LISTED ARE FOR MAXIMUM YIELDS.

5.1 Cell lysis

Before you start, please read the information section relevant to your sample type (Section 6.1 Leaf extractions, Section 6.2 Seed extractions).

1. Grind dry tissue samples until homogenised; a maximum of 20 - 30 mg material is recommended. For fresh or frozen samples start immediately with the second step.
2. Add 75 µL buffer L1 to the ground sample and mix well.
3. Incubate the samples:
    - If processing leaf material or seed material without the addition of α-amylase to L1, incubate at 55°C for 10 minutes.
    - If processing seed material with the addition of α-amylase to L1, incubate at 91°C for 30 minutes in a waterbath.
4. Add 150 µL buffer C1 to each lysed sample, and mix until the solution is homogenous.
5. Incubate samples at 55°C for 10 minutes.
6. Centrifuge your samples at 3,000 *x* g for 2 minutes to clear the mixtures of most cellular debris.

5.2 Absorption of DNA to glass fibre membranes

7. Place the filter plate on top of another deep-well plate or reservoir:
    - 96-well filter plate kits: Use a plate with a capacity of at least 300 µL / well or an equivalent reservoir
    - 384-well filter plate kits: Use a plate with a capacity of at least 200 µL / well or an equivalent reservoir

8. Transfer the cleared lysate / binding buffer mixture into the wells of the filter plate, ensuring that as little debris as possible is transferred along with the cleared lysate; excess debris may block the membranes in the filter.
    - 96-well filter plate kits: The entire mixture can be transferred if it is available.
    - 384-well filter plate kits: There is insufficient capacity in the wells of the filter plate to allow the entire mixture to be transferred in one go; 100 µL of the mixture should be transferred (the rest of the mixture can be extracted using the same filter plate at a later date, if required; see the 'Notes' section for details regarding this).
9. Incubate the filter plates for 2 minutes at room temperature, then centrifuge the filter plate on top of the deep-well collection plate or reservoir at 3,000 *x* g for 2 minutes.
10. Clear spin through from the collection plate / reservoir, and place the filter plate back on top of it.

5.3 Contaminant removal through washing

11. Add buffer A1 to each sample well on the filter plate:
    - 96-well filter plate kits: Add 150 µL
    - 384-well filter plate kits: Add 75 µL
12. Incubate the filter plates for 2 minutes at room temperature, then centrifuge the filter plate on top of the deep-well collection plate or reservoir at 3,000 *x* g for 2 minutes.
13. Clear spin through from the collection plate / reservoir, and place the filter plate back on top of it.
14. Add buffer W1 to each sample well on the filter plate:
    - 96-well filter plate kits: Add 75 µL
    - 384-well filter plate kits: Add 37.5 µL
15. Incubate the filter plates for 2 minutes at room temperature, then centrifuge the filter plate on top of the deep-well collection plate or reservoir at 3,000 *x* g for 2 minutes.
16. Clear spin through from the collection plate / reservoir, and place the filter plate back on top of it.
17. Add 100% ethanol to each sample well on the filter plate:
    - 96-well filter plate kits: Add 75 µL
    - 384-well filter plate kits: Add 37.5 µL

18. Incubate the filter plates for 2 minutes at room temperature, then centrifuge the filter plate on top of the deep-well collection plate or reservoir at 3,000 *x* g for 2 minutes.
19. Incubate the filter plates on their own for 10 minutes at 50 - 70°C to eliminate any residual ethanol.

5.4 Elution of purified DNA

20. Replace the deep-well collection plate / reservoir used up to this point with an elution plate, and place the filter plate back on top of it.
21. Add buffer E1 (pre-warmed to 55°C) to each sample well on the filter plate:
    N.B. - Yield is significantly reduced if the elution buffer is not pre-warmed to 55°C
    - 96-well filter plate kits: Add 75 µL
    - 384-well filter plate kits: Add 37.5 µL
22. Incubate at 55°C for 5 minutes, then centrifuge the filter plate on top of the deep-well collection plate at 3,000 *x* g for 2 minutes to elute the DNA.

6. General points

The protocol is applicable in principle to any plant, however the protocol was developed and tested on the following sources:

- Barley (*Hordeum vulgare*)
- Cowpea (*Vigna unguiculata*)
- Lettuce (*Lactuca sativa*)
- Maize (*Zea mays*, leaves and kernels)
- Soy (*Glycine max L.*)
- Sugar beet (*Beta vulgaris*)
- Sweet pepper (*Capsicum annuum*, leaves and seeds)
- Tomato (*Solanum lycopersicum*, leaves and seeds)
- Wheat (*Triticum aestivum*, leaves and seeds).

DNA is not significantly fragmented by the method, with fragment sizes of > 50 kb typically seen. The absence of nucleases in samples prepared with Kleargene has been demonstrated by overnight incubation at 37°C in the presence of 10 mM $MgCl_2$.

6.1 Leaf extractions

- The protocol assumes you have lyophilised or dessicated your samples before extraction; this allows you to homogenise the samples 'dry' before extraction, which is most efficient and allows you to extract from relatively more sample than would otherwise be the case (up to 30 mg).
- Drying samples in an oven is not recommended as the heat degrades the sample.
- When processing very small amounts of sample (e.g. 1 or 2 punches), it is possible to homogenise the sample in buffer L1 rather than beforehand; this allows you to handle samples without prior drying.
- Note that if the homogenisation is carried out in buffer L1 then overloading of sample will lead to inefficient disruption and lower yields downstream. Younger and fresher source leaves give the best results.
- Seedlings can be used, although monocots may prove tougher to disrupt fully.
- Etiolated samples are not generally required; the compositions of the buffers used are sufficient to deal with the metabolites normally found in leaf material.

6.2 Seed extractions

- Extracting DNA from seeds presents a slightly different challenge to extracting DNA from leaves; the concentration of potentially interfering metabolites is much lower, but the presence of starch can cause blockages to the membranes of filter plates.
    - Seeds are highly variable in size, and in the case of smaller seeds (e.g. tomato, pepper) careful aspiration of the L1 / C1 lysate mixture will probably be OK as it should be possible to avoid transferring significant amounts of starch over to the filter plate this way.
    - For bigger seeds (e.g. corn kernels) the amounts of starch produced after grinding will cause significant blocking issues downstream; in these instances, it is recommended that the enzyme α-amylase is added to buffer L1. The α-amylase degrades insoluble starch into a mixture of dextrins that are soluble in L1, thus allowing the cleared lysate to pass through the filter plate membrane.

6.3 Adapting the protocol to your laboratory

- If necessary, the extraction process can be suspended after any washing step and the plates can be kept at room temperature for many hours.
- If the protocol is halted for an extended period of time at any point before step 7, the lysate mixtures should be refrigerated to limit the oxidation of the plant compounds that have been released into solution.
    - If this is done, it is vital that the mixtures are thoroughly warmed and mixed well before continuing the process, as certain buffer components are liable to form precipitates.

- When using the 384-well filter plates, the well capacity of the filter plates limits the volume of sample that can be processed at one time.
    - The protocol above specifies how ~100 µL of the L1 / C1 lysate mixture should be extracted. If additional yield is required, it is possible to repeat the extraction process using the same filter plate after the initial elution has been carried out (be careful to ensure the correct orientation of the filter plate and samples if this is done).
- A single elution typically yields 70 - 80% of the total DNA bound to the glass fibre in the filter plates; a second elution step can be carried out to remove the majority of all the remaining DNA if maximal yield is required. This will inevitably reduce the overall concentration of the eluted DNA.
- You are supplied with an approximate two-fold excess of each buffer over the volumes needed to carry out an extraction exactly as defined in the protocol. This allows you flexibility at certain stages of the protocol. The ratios of buffers used must be maintained:

  1 volume L1 : 2 volumes C1 : 2 volumes A1 : 1 volume W1 : 1 volume EtOH: 1 volume E1

    - Many plant materials occlude a certain proportion of buffer L1 such that not all 75 µL is available for downstream processing. It is acceptable to add additional L1 initially. However, if this is done, you **must** also increase the volumes of all other buffers in proportion. The protocol is likely to fail if you do not do this.

7. Troubleshooting

Problem	Likely cause	Explanation / suggestions
Blocked filter plate	Excess starch in lysate	Add α-amylase to buffer L1 before use (seeds only)
	Excessive starting material	Use less starting material in future extractions.
Low DNA yield	Insufficient disruption of tissue prior to addition of buffer L1	Ensure that tissue samples are ground / homogenised before use.
	Insufficient lysis	Ensure that the amount of tissue used is within the recommended range (up to 30 mg dry tissue). Excess starting material will reduce the ability of cells to lyse. Insufficient starting material will result in lower than expected yields.
	Buffer E1 not pre-warmed to 55°C before use	DNA elution from the filter plate is much more efficient when buffer E1 is at 55°C
DNA is degraded	Poor sample storage prior to extraction	Ensure that tissues are stored appropriately to minimise DNA degradation. It is recommended that all plant tissue should be stored at -20°C or lower. Younger and fresher source tissue samples may produce better quality DNA.
	Tissue samples were dried in an oven	Oven drying of samples is not recommended as heat degrades plant tissue. Lyophilise tissue for storage prior to performing DNA extraction, or grind up fresh tissue in liquid nitrogen and store at -80°C.
	Precipitate has formed in buffer L1 (lysis buffer)	Incubate buffer at 37°C and mix well until clear.
DNA does not perform well in downstream experiments	Ethanol carryover	Ensure that the spin plate is completely dry after the final wash step with ethanol.
	Salt carryover	Ensure that the wash buffers (A1 and W1) are at room temperature before use.
	Insufficient / excessive DNA used in downstream experiments.	Optimise the quantity of DNA that is used in downstream experiments with a DNA dilution series. Too much or too little DNA can adversely affect experimental performance.

8. Frequently asked questions

1. What type of centrifuge is required for the Kleargene plant tissue protocol?

The centrifuge will need an adapter suitable for plates, and to be capable of achieving 3000 *x* g.

2. Can the Kleargene buffers be purchased individually?

Yes. All buffers can be purchased individually.

Buffer	Volume	Product code
L1	250 mL	KBS-1012-306
C1	500 mL	KBS-1012-308
A1	250 mL	KBS-1012-309
W1	75 mL	KBS-1012-310
E1	250 mL	KBS-1012-311

Additional filter plates can also be purchased

Plate size	Pack size	Product code
96-well	10 plates	KBS-1012-303
384-well	10 plates	KBS-1012-304

3. Can the Kleargene kit be used for the extraction of RNA from plant tissue?

No.

If you are working with large numbers of samples, why not consider our Genespin™ platform. The Genespin enables semi-automated, high-throughput DNA extractions from plant tissues and utilises Kleargene chemistry.

For any queries about this guide please contact:
All locations except USA: email tech.support@lgcgroup.com or call +44 (0)1992 476 486
USA only: email us-support@lgcgroup.com or call +1 978 338 5317

www.lgcgroup.com/genomics • genomics@lgcgroup.com

Science for a safer world

Brazil • Bulgaria • China • Czech Republic • Finland • France • Germany • Hungary • India • Ireland • Italy • Netherlands
Poland • Romania • Russia • South Africa • Spain • Sweden • Turkey • United Kingdom • USA