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Handbook for
GenEx™ Plant
GenEx™ Plant *plus!*

DNA PURIFICATION HANDBOOK

genex tm

GeneAll

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This protocol handbook is included in :

GeneAll® *GenEx*™ Plant (Cat. No. 227-101, 227-201, 227-301)
GeneAll® *GenEx*™ Plant *plus!* (Cat. No. 228-101, 228-250, 228-320)

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

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GenEx™ Plant

Cat.No.	227-101	227-201	227-301
Size	Sx	Mx	Lx
Standard sample weight(wet)	100 mg	500 mg	2 g
No. of preparation	100	100	100
Buffer PL	50 ml	250 ml	1 L
Buffer PP	14 ml	70 ml	270 ml
Buffer RE	30 ml	110 ml	220 ml
RNase A solution (100 mg/ml)	300 ul	1.5 ml	6 ml
Protocol Handbook	1	1	1

GenEx™ Plant *plus!*

Cat.No.	228-101	228-250	228-320
Size	Sx	Mx	Lx
Standard sample weight(wet)	100 mg	500 mg	2 g
No. of preparation	100	50	20
Buffer PL	50 ml	125 ml	200 ml
Buffer PP	14 ml	35 ml	55 ml
Buffer RE	30 ml	60 ml	60 ml
RNase A solution (100 mg/ml)	300 ul	750 ul	1.2 ml
EzSep™ Filter Column	100ea (mini)	50ea (Midi)	20ea (MAXI)
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Product Disclaimer

GeneAll® *GenEx*™ Plant kit is for research use only, and should not be used for drug, household or other unintended uses. All due care and attention should be taken in every procedure in this handbook. Please consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Storage and Stability

GeneAll® *GenEx*™ Plant kit is shipped at ambient condition. Basically all components are stable at room temperature (15 ~ 25°C). But for enzyme, RNase A, it is recommended to store under 4°C for prolonged activity. A precipitate can be formed in buffer PL under cool ambient condition. In such a case, heat the bottle at 56°C until completely dissolving before use.

Safety Information

Buffer PL and PP contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow the standard safety precautions.

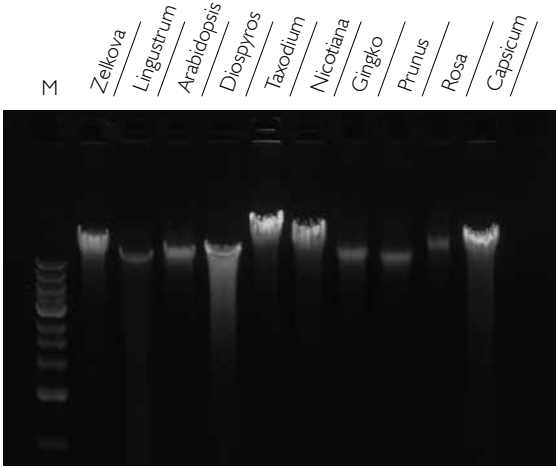
Quality Control

All components in GeneAll® *GenEx*™ Plant kit are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. Restriction enzyme assay, PCR amplification assay and spectrophotometric assay as the validation of quality are carried out from lot to lot thoroughly, and only the qualified is approved to deliver.

Introduction

GeneAll® *GenEx*™ Plant kit provides an easy and convenient method for the isolation of total DNA from various plant samples without use of toxic chemical such as phenol or chloroform. This kit has a specially formulated solution format and enables the scalable preparation of almost intact size DNA. Especially when purifying DNA from plant, the removal of secondary metabolites is very important because contamination of these impurities can lead to inhibition of downstream application. The optimized buffer system adopted in this kit can facilitate the removal of contaminants, such as second metabolites and other impurities. Purified DNA can be applied directly to PCR, blotting, restriction enzyme assay and other downstream applications.

GeneAll® *GenEx*™ Plant *plus!* kit has an additional feature, EzSep™ filter column. With certain plant samples, it is very difficult to separate cleared supernatant from pelletal debris at a protein precipitation stage. This problem also appears often when large starting sample and it may be due to low density of debris and/or low centrifugal force with conventional centrifuge. EzSep™ filter column included in the *plus!* kit is the device to solve this problem and moreover it decreases the preparation time also.



Total DNA prepared from various plant leaves using *GenEx*™ Plant kit. Each sample is extracted from 100 mg of tissue approximately. And 4 uls of purified DNA were resolved on 1.0 % agarose gel. M; 1 kb DNA ladder

GenEx™ Plant kit procedures

General Considerations

Sample preparations, pulverizations and lysis

When purifying total DNA from plant samples, harvest and pulverization are the critical steps for good result. Harvested plant sample should be used directly for preparation or stored under -70°C immediately after frozen in liquid nitrogen for future use. Lyophilized tissue can be stored at room temperature. Use of young and fresh sample is always best for optimal result.

The starting tissue sample should be completely disrupted for efficient lysis and this should be performed at low temperature as quickly as possible. Mortar and pestle with liquid nitrogen is a typical and good method for disrupting of plant sample, while rotor-stator homogenizer or bead-beater can be a good alternative. Complete and quick pulverization will guarantee the optimized result, while incomplete disrupting, delayed thawing or mishandling of sample may lead to poor result, such as low yield or degradation. Lyophilized tissue can be ground at ambient condition.

Sample mixture should be homogenized without any clumps after addition of buffer PL. Because the clumped sample will not be lysed properly and lead to a poor result. Vortexing or pipetting should be carried out for good result. Incubating at 65°C for 20~30 minutes will be sufficient for typical preparations from leaf tissue, but this lysis time can be prolonged depending on the tissue type used. Periodical mixing will accelerate the lysis efficiency.

Removal of contaminants

Many unwanted components included in cell lysate, such as proteins, carbohydrates, lipids and secondary metabolites should be removed from the preparation, and it can be done by precipitation. These impurities can be removed by several methods such as the decreasing of solubility by salting out or pH alteration and specific precipitation. Buffer PP induces the precipitation of contaminants by combined effect without use of harmful organic solvent.

With certain plant sample, it is very difficult to separate cleared supernatant from the debris of impurities by centrifugation. This problem also appears often when large starting sample. It may be due to low density of debris and/or low centrifugal force. EzSep™ filter column is a convenient device to solve it and moreover it reduces the preparation time also. EzSep™ filter column is included in *GenEx*™ Plant *plus!* kit and it is also available to purchase separately

DNA precipitating

Alcohol precipitation is a usual method to concentrate nucleic acid, and it can be achieved by addition of 2 volumes of ethanol or 0.6 volumes of isopropanol in the presence of mono cation. Alcohol removes hydration shell of DNA and then uncovers phosphate group which has negative charge. Uncovered phosphate group is neutralized by positive ion, such as Na^+, followed by precipitation of DNA due to the loss of solubility to water.

When the starting sample is very small, the consequent yield will be very low. It is because the precipitation of DNA can not be taken place properly when small concentration of DNA. In this case, some nucleic acid carrier, such as tRNA or glycogen, should be added before addition of alcohol. Precipitated DNA is washed by 70% ethanol and air-dried before rehydration with buffer RE or water.

DNA Rehydration

Precipitated DNA pellet can be rehydrated by low salt buffer or deionized destilled water depending on the downstream application. Buffer RE contains 1 mM EDTA and the pH is adjusted to 8.0 with Tris/HCl. Water can be also used but it is not recommended for long-term storage because it lacks the ability of DNA stabilization. Over-drying of DNA pellet after ethanol washing will make the rehydration very difficult. In certain plant sample, it is very difficult to remove the unwanted components completely and these contaminants can also disturb the rehydration of DNA pellet.

Normally, rehydration of DNA will be accomplished in an hour. Alternatively, rehydration can be carried out at 4°C overnight.

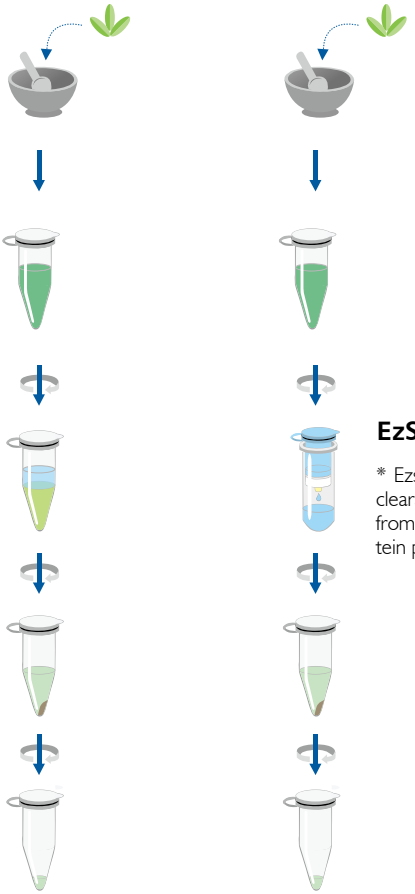
The amount used per sample weight

Sample weight		Buffer PL	RNase A	Buffer PP	Isopropanol	70% Ethanol	Buffer RE	Tube size
Wet	Dried							
100 mg	25 mg	500 ul	3 ul	140 ul	300 ul	300 ul	100 ul	1.5 ml
200 mg	50 mg	1 ml	6 ul	270 ul	600 ul	600 ul	100 ul	1.5 ml
300 mg	75 mg	1.5 ml	9 ul	400 ul	900 ul	900 ul	100 ul	15 ml
400 mg	100 mg	2 ml	12 ul	540 ul	1.2 ml	1.2 ml	200 ul	15 ml
500 mg	125 mg	2.5 ml	15 ul	670 ul	1.5 ml	1.5 ml	200 ul	15 ml
600 mg	150 mg	3 ml	18 ul	800 ul	1.7 ml	1.7 ml	200 ul	15 ml
800 mg	200 mg	4 ml	24 ul	1.1 ml	2.3 ml	2.3 ml	200 ul	15 ml
1,000 mg	250 mg	5 ml	30 ul	1.4 ml	3 ml	3 ml	300 ul	15 ml
1,200 mg	300 mg	6 ml	36 ul	1.6 ml	3.5 ml	3.5 ml	300 ul	50 ml
1,500 mg	375 mg	7.5 ml	45 ul	2 ml	4.5 ml	4.5 ml	300 ul	50 ml
2,000 mg	500 mg	10 ml	60 ul	2.7 ml	6 ml	6 ml	500 ul	50 ml
3,000 mg	750 mg	15 ml	90 ul	4 ml	9 ml	9 ml	500 ul	50 ml

This table represents the common value for preparations. The amount of the solution can be varied depending on the species used.

Brief Procedures

	GenEx™ Plant	*GenEx*™ Plant *plus!*
Homogenization		
Lysis step		
Protein precipitation		
DNA precipitation		
Pure DNA		



EzSep™ filter

* Ezsep™ filter facilitates the clearance of the supernatant from pelletal debris at a protein precipitation stage

GenEx™ Plant Protocol – for 100 mg of plant tissue

Additional equipments or materials to be supplied by user

Sterile 1.5 ml microcentrifuge tubes
Water bath or heat block at 65°C
Ice (optional)
Isopropanol
70% ethanol

* Buffer PL may precipitate at cool ambient temperature. If so, dissolve it completely in 65°C water bath.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place 100 mg (wet) or 25 mg (dried) of ground sample into a 1.5 ml tube.

Quick and complete pulverization is essential for good result in preparation. Grinding under liquid nitrogen is a good method for most plant samples, however other method such as bead-beater or rotor-stator homogenizer can be a good alternative.
Lyophilized tissue sample can be ground at room temperature.

2. Add 500 ul of Buffer PL and 3 ul of RNase A into the tube and vortex vigorously to mix homogeneously.

3. Incubate the mixture at 65°C for 15 min.

Periodical mixing by vortexing will accelerate the lysis.

4. Centrifuge the lysate for 30 sec at 14,000 xg and transfer the 400 ul of supernatant into a new 1.5 ml tube.

Co-transfer of some debris can be occurred and it has no influence on preparation.

Transfer it as much as possible when the volume of supernatant is lower than 400 ul.

5. Add 140 ul of Buffer PP to the lysate and vortex vigorously for 15 sec. Centrifuge for 5 min at 14,000 xg.

***(Optional)* Incubate the sample on ice for 5 min before centrifugation. This may slightly increase the quality of DNA.**

If the volume of lysate transferred at step 4 is lower than 400 ul, adjust the volume of buffer PP to 1/3 volume of the transferred lysate.

6. Carefully transfer 400 ul of the supernatant by pipetting to the fresh 1.5 ml tube containing 300 ul of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

Be careful not to co-transfer the pellet or debris. The pellet can be formed loosely depending on the species of starting sample. In such a case, special care should be taken place.

Isopropanol can be added later.

Do not vortex after addition of isopropanol.

The white thread-like strands can be indistinct or invisible depending on the mass of DNA.

- Clear separation between the supernatant and the pellet can't be taken place with the tissue of certain species because of the density of debris. If the supernatant is not clear after centrifuge and correct transfer is not available, EzSep™ mini filter should be used with the protocol at page 21.
- *GenEx*™ Plant *plus!* kit with EzSep™ mini filter is available or EzSep™ mini filter can be purchased separately.

7. **Centrifuge at 14,000 xg for 1 min. Discard the supernatant and add 300 ul of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

   Pellet can be slightly colored due to some remaining contaminants.

8. **Centrifuge at 14,000 xg for 1 min. Discard the ethanol carefully by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 5 ~ 10 min.**

   The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

   Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.

9. **Add 100 ul of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 20 min or at RT for 1 hour.**

   During incubation, periodically mix the DNA solution by gently tapping the tube.

   DNA can be rehydrated alternatively by incubating the solution overnight at 4°C.

GenEx™ Plant Protocol – for 500 mg of plant tissue

Additional equipments or materials to be supplied by user

Sterile 15 ml conical tubes
Water bath at 65°C
Ice (optional)
Isopropanol
70% ethanol

* Buffer PL may precipitate at cool ambient temperature. If so, dissolve it completely in 65°C water bath.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place 500 mg (wet) or 125 mg (dried) of ground sample into a 15 ml tube.

Quick and complete pulverization is essential for good result in preparation. Grinding under liquid nitrogen is a good method for most plant samples, however other method such as bead-beater or rotor-stator homogenizer can be a good alternative.

Lyophilized tissue sample can be ground at room temperature.

2. Add 2.5 ml of Buffer PL and 15 ul of RNase A into the tube and vortex vigorously to mix homogeneously.

3. Incubate the mixture at 65°C for 20 min.

Periodical mixing by vortexing will accelerate the lysis.

4. Centrifuge the lysate for 3 min at 3,000 xg and transfer the 2 ml of supernatant into a new 15 ml tube.

Co-transfer of some debris can be occurred and it has no influence on preparation.

Transfer the supernatant as much as possible when the volume is lower than 2 ml.

5. Add 670 ul of Buffer PP to the mixture and vortex vigorously for 20 sec. Centrifuge for 10 min at 3,000 xg.
(Optional) Incubate the sample on ice for 5 min before centrifugation. This may slightly increase the quality of DNA.

If the volume of lysate transferred at step 4 is lower than 2 ml, adjust the volume of buffer PP to 1/3 volume of the lysate.

6. Carefully transfer 2 ml of the supernatant by pipetting to the fresh 15 ml tube containing 1.5 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

Be careful not to co-transfer the pellet or debris. The pellet can be formed loosely depending on the species of starting sample. In such a case, special care should be taken place.

Isopropanol can be added later.

Do not vortex after addition of isopropanol.

The white thread-like strands can be indistinct or invisible depending on the mass of DNA.

- Clear separation between the supernatant and the pellet can't be taken place with the tissue of certain species because of the density of debris. If the supernatant is not clear after centrifuge and correct transfer is not available, EzSep™ Midi filter should be used with the protocol at page 24.
- *GenEx*™ Plant *plus!* kit with EzSep™ Midi filter is available or EzSep™ Midi filter can be purchased separately.

7. **Centrifuge at 3,000 xg for 3 min. Discard the supernatant and add 1.5 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

   Pellet can be slightly colored due to some remaining contaminants.

8. **Centrifuge at 3,000 xg for 3 min. Discard the ethanol carefully by aspirating or decanting. Invert the tube on clean absorbent paper and air-dry the pellet for 5 ~ 10 min.**

   The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

   Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.

9. **Add 200 ul of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 20 min or at RT for 1 hour.**

   During incubation, periodically mix the DNA solution by gently tapping the tube.

   DNA can be rehydrated alternatively by incubating the solution overnight at 4°C.

GenEx™ Plant Protocol – for 2 g of plant tissue

Additional equipments or materials to be supplied by user

Sterile 50 ml conical tubes
Water bath at 65°C
Ice (optional)
Isopropanol
70% ethanol

* Buffer PL may precipitate at cool ambient temperature. If so, dissolve it completely in 65°C water bath.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place up to 2 g (wet) or 400 mg (dried) of ground sample into a 50 ml tube.

Quick and complete pulverization is essential for good result in preparation. Grinding under liquid nitrogen is a good method for most plant samples, however other method such as bead-beater or rotor-stator homogenizer can be a good alternative.

Lyophilized tissue sample can be ground at room temperature.

2. Add 10 ml of Buffer PL and 60 ul of RNase A into the tube and vortex vigorously to mix homogeneously.

3. Incubate the mixture at 65°C for 30 min.

Periodical mixing by vortexing will accelerate the lysis.

4. Centrifuge the lysate for 3 min at 3,000 xg and transfer the 8 ml of supernatant into a new 50 ml tube.

Co-transfer of some debris can be occurred and it has no influence on preparation.

Transfer the supernatant as much as possible when the volume is lower than 8 ml.

5. Add 2.7 ml of Buffer PP to the mixture and vortex vigorously for 20 sec. Centrifuge for 10 min at 3,000 xg.
(Optional) Incubate the sample on ice for 10 min before centrifugation. This may slightly increase the quality of DNA.

If the volume of lysate transferred at step 4 is lower than 8 ml, adjust the volume of buffer PP to 1/3 volume of the lysate.

6. Carefully transfer the supernatant by pipetting to the fresh 50 ml tube containing 6 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

Be careful not to co-transfer the pellet or debris. The pellet can be formed loosely depending on the species of starting sample. In such a case, special care should be taken place.

Isopropanol can be added later.

Do not vortex after addition of isopropanol.

The white thread-like strands can be indistinct or invisible depending on the mass of DNA.

- Clear separation between the supernatant and the pellet can't be taken place with the tissue of certain species because of the density of debris. If the supernatant is not clear after centrifuge and correct transfer is not available, EzSep™ MAXI filter should be used with the protocol at page 27.
- *GenEx*™ Plant *plus!* kit with EzSep™ MAXI filter is available or EzSep™ MAXI filter can be purchased separately.

7. **Centrifuge at 3,000 xg for 3 min. Discard the supernatant and add 6 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

   Pellet can be slightly colored due to some remaining contaminants.

8. **Centrifuge at 3,000 xg for 3 min. Discard the ethanol carefully by aspirating or decanting. Invert the tube on clean absorbent paper and air-dry the pellet for 5 ~ 10 min.**

   The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

   Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.

9. **Add 500 ul of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 20 min or at RT for 1 hour.**

   During incubation, periodically mix the DNA solution by gently tapping the tube.

   DNA can be rehydrated alternatively by incubating the solution overnight at 4°C.

GenEx™ Plant *plus!* Protocol with EzSep™ mini filter – for 100 mg of plant tissue

This protocol requires GenEx Plant Sx plus! Kit.
Additional equipments or materials to be supplied by user

Sterile 1.5 ml microcentrifuge tubes
Water bath or heat block at 65°C
Ice (optional)
Isopropanol
70% ethanol

* Buffer PL may precipitate at cool ambient temperature. If so, dissolve it completely in 65°C water bath.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place 100 mg (wet) or 25 mg (dried) of ground sample into a 1.5 ml tube.

Quick and complete pulverization is essential for good result in preparation. Grinding under liquid nitrogen is a good method for most plant samples, however other method such as bead-beater or rotor-stator homogenizer can be a good alternative.
Lyophilized tissue sample can be ground at room temperature.

2. Add 500 ul of Buffer PL and 3 ul of RNase A into the tube and vortex vigorously to mix homogeneously.

3. Incubate the mixture at 65°C for 15 min.

Periodical mixing by vortexing will accelerate the lysis.

4. **Centrifuge the lysate for 30 sec at 14,000 xg and transfer the 400 ul of supernatant into a new 1.5 ml tube.**
   Co-transfer of some debris can be occurred and it has no influence on preparation.
   Transfer it as much as possible when the volume of supernatant is lower than 400 ul.

5. **Add 140 ul of Buffer PP to the lysate and vortex vigorously for 15 sec.**
   If the volume of lysate transferred at step 4 is lower than 400 ul, adjust the volume of buffer PP to 1/3 volume of the transferred lysate.

6. **Apply all of the mixture into an EzSep™ mini filter column and centrifuge for 2 min at 14,000 xg.**
   The mixture can be flowed by gravity through a membrane before centrifugation.

7. **Carefully transfer 400 ul of the supernatant by pipetting to the fresh 1.5 ml tube containing 300 ul of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   Be careful not to co-transfer the pellet or debris. The pellet can be formed loosely depending on the species of starting sample.
   Isopropanol can be added later.
   Do not vortex after addition of isopropanol.
   The white thread-like strands can be indistinct or invisible depending on the mass of DNA.

8. Centrifuge at 14,000 xg for 1 min. Discard the supernatant and add 300 ul of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.

Pellet can be slightly colored due to some remaining contaminants.

9. Centrifuge at 14,000 xg for 1 min. Discard the ethanol carefully by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 5 ~ 10 min.

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.

10. Add 100 ul of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 20 min or at RT for 1 hour.

During incubation, periodically mix the DNA solution by gently tapping the tube.

DNA can be rehydrated alternatively by incubating the solution overnight at 4°C.

GenEx™ Plant *plus!* Protocol with EzSep™ Midi filter – for 500 mg of plant tissue

This protocol requires GenEx™ Plant Mx plus! Kit.
Additional equipments or materials to be supplied by user

Sterile 15 ml conical tubes
Water bath at 65°C
Ice (optional)
Isopropanol
70% ethanol

* Buffer PL may precipitate at cool ambient temperature. If so, dissolve it completely in 65°C water bath.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place 500 mg (wet) or 125 mg (dried) of ground sample into a 15 ml tube.

Quick and complete pulverization is essential for good result in preparation. Grinding under liquid nitrogen is a good method for most plant samples, however other method such as bead-beater or rotor-stator homogenizer can be a good alternative.

Lyophilized tissue sample can be ground at room temperature.

2. Add 2.5 ml of Buffer PL and 15 ul of RNase A into the tube and vortex vigorously to mix homogeneously.

3. Incubate the mixture at 65°C for 20 min.

Periodical mixing by vortexing will accelerate the lysis.

4. **Centrifuge the lysate for 3 min at 3,000 xg and transfer the 2 ml of supernatant into a new 15 ml tube.**
   Co-transfer of some debris can be occurred and it has no influence on preparation.
   Transfer the supernatant as much as possible when the volume is lower than 2 ml.

5. **Add 670 ul of Buffer PP to the mixture and vortex vigorously for 20 sec.**
   If the volume of lysate transferred at step 4 is lower than 2 ml, adjust the volume of buffer PP to 1/3 volume of the lysate.

6. **Apply all of the mixture into an EzSep™ Midi filter column, close the cap, and centrifuge for 5 min at 3,000 xg.**
   The mixture can be flowed by gravity through a column membrane before centrifugation.

7. **Carefully transfer 2 ml of the supernatant by pipetting or decanting to the fresh 15 ml tube containing 1.5 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   Be careful not to co-transfer the pellet or debris. The pellet can be formed loosely depending on the species of starting sample.
   Isopropanol can be added later.
   Do not vortex after addition of isopropanol.
   The white thread-like strands can be indistinct or invisible depending on the mass of DNA.

8. **Centrifuge at 3,000 xg for 3 min. Discard the supernatant and add 1.5 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

   Pellet can be slightly colored due to some remaining contaminants.

9. **Centrifuge at 3,000 xg for 3 min. Discard the ethanol carefully by aspirating or decanting. Invert the tube on clean absorbent paper and air-dry the pellet for 5 ~ 10 min.**

   The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

   Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.

10. **Add 200 ul of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 20 min or at RT for 1 hour.**

    During incubation, periodically mix the DNA solution by gently tapping the tube.

    DNA can be rehydrated alternatively by incubating the solution overnight at 4°C.

GenEx™ Plant *plus!* Protocol with EzSep™ MAXI filter – for 2 g of plant tissue

This protocol requires GenEx™ Plant Lx plus! Kit.
Additional equipments or materials to be supplied by user

Sterile 50 ml conical tubes
Water bath at 65°C
Ice (optional)
Isopropanol
70% ethanol

* Buffer PL may precipitate at cool ambient temperature. If so, dissolve it completely in 65°C water bath.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place up to 2 g (wet) or 400 mg (dried) of ground sample into a 50 ml tube.

Quick and complete pulverization is essential for good result in preparation. Grinding under liquid nitrogen is a good method for most plant samples, however other method such as bead-beater or rotor-stator homogenizer can be a good alternative.

Lyophilized tissue sample can be ground at room temperature.

2. Add 10 ml of Buffer PL and 60 ul of RNase A into the tube and vortex vigorously to mix well.

3. Incubate the mixture at 65°C for 30 min.

Periodical mixing by vortexing will accelerate the lysis.

4. **Centrifuge the lysate for 3 min at 3,000 xg and transfer the 8 ml of supernatant into a new 50 ml tube.**
   Co-transfer of some debris can be occurred and it has no influence on preparation.
   Transfer the supernatant as much as possible when the volume is lower than 8 ml.

5. **Add 2.7 ml of Buffer PP to the mixture and vortex vigorously for 20 sec.**
   If the volume of lysate transferred at step 4 is lower than 8 ml, adjust the volume of buffer PP to 1/3 volume of the lysate.

6. **Apply all of the mixture into an EzSep™ MAXI filter column, close the cap, and centrifuge for 5 min at 3,000 xg.**
   The mixture can be flowed by gravity through a column membrane before centrifugation.

7. **Carefully transfer 8 ml of the supernatant by pipetting or decanting to the fresh 50 ml tube containing 6 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   Be careful not to co-transfer the pellet or debris. The pellet can be formed loosely depending on the species of starting sample. In such a case, special care should be taken place.
   Isopropanol can be added later.
   Do not vortex after addition of isopropanol.
   The white thread-like strands can be indistinct or invisible depending on the mass of DNA.

8. Centrifuge at 3,000 xg for 3 min. Discard the supernatant and add 6 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.

Pellet can be slightly colored due to some remaining contaminants.

9. Centrifuge at 3,000 xg for 3 min. Discard the ethanol carefully by aspirating or decanting. Invert the tube on clean absorbent paper and air-dry the pellet for 5 ~ 10 min.

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.

10. Add 500 ul of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 20 min or at RT for 1 hour.

During incubation, periodically mix the DNA solution by gently tapping the tube.

DNA can be rehydrated alternatively by incubating the solution overnight at 4°C.

Trouble shooting

Facts	Possible Causes	Suggestions
Low or no yield	Too much starting sample	Use of too much sample can lead to inefficient lysis followed by poor yield. Decrease the starting sample.
	Too old or mis-stored starting sample	Generally, the best result will be obtained from young fresh sample. DNA will be degraded gradually during storage, and improper condition can accelerate the breakdown.
	Insufficient pulverization	For best result, tissue sample should be pulverized completely using a proper method, such as mortar and pestle.
	Cell clumps present in the lysate	The clumps present in the lysate will not be lysed efficiently. Homogenize by vortexing or pipetting before incubation.
	Low cells in the starting sample	Some plant sample may contain low number of cells per weight because of its high water composition. Increase the starting sample or dehydrate before weighing.
	Lost DNA pellet during precipitation	Intensive care should be taken in removing the isopropanol and the ethanol not to lose the DNA pellet during precipitation procedures.
	DNA pellet is not completely rehydrated	Rehydrate the DNA by incubating for 1 hour at 65°C and then leave it at room temperature or 4°C overnight. Do not leave the DNA solution at 65°C overnight. DNA may be degraded.
Low purity	Too much starting sample	Too much starting sample can lead to poor lysis, followed by low purity.
	Incorrect transfer of cleared supernatant	After centrifugation of PP-added mixture, cleared supernatant should be transferred to a fresh tube without any debris or precipitates.

Facts	Possible Causes	Suggestions
Supernatant not clear after protein precipitation	With certain plant species, the supernatant after protein precipitation can't be easily cleared by centrifugation because of its chemical compositon.	Use of EzSep™ filter column facilitates the clearing of supernatant. *GenEx*™ Plant *plus!* kit with EzSep™ filter is available or EzSep™ filter can be purchased separately.
Clogging of EzSep™ filter	Low centrifugal force	Increase the g-force and centrifugation time.
Degraded DNA	Too much starting sample	Too much starting sample can make the lysate very viscous and lead to shearing of DNA. Reduce the starting sample.
	Too old or mis-stored sample	Generally, the best result will be obtained from young fresh sample. DNA will be degraded gradually during storage, and improper condition can accelerate the breakdown.
	Over-handling of sample	DNA can be sheared due to over-handling, such as over-pulverizing of the starting tissue or pipetting of the DNA rehydrate.
	Too viscous lysate	DNA can be sheared in viscous lysate. Extra addition of buffer PL may reduce the viscousity.
DNA pellet difficult to dissolve	Over dried pellet	DNA pellet should not be dried for longer than 15 min at room temperature. Rehydrate the DNA by incubating for 1 hour at 65°C and then leave it at room temperature or 4°C overnight. Do not leave the DNA solution at 65°C overnight. DNA may be degraded.
Colored residue in DNA solution	Too much starting sample	Too much starting sample can lead to poor lysis, followed by colored residue in the DNA.
	Insufficient protein precipitation	After centrifugation of PP-added mixture, cleared supernatant should be transferred to a fresh tube without any debris or precipitates. Co-transfer of debris will bring on the colored residue of DNA.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep		50	100-150	mini / spin
		100	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
		1,000	101-111	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™*
for preparation of highly pure plasmid DNA

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue *plus!* SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
GMO SV	mini	50	107-150	spin / vacuum
		200	107-102	

GeneAll® *GenEx™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type

GeneAll® *GenEx™* *for isolation of total DNA*

GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant *plus!*	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	

GeneAll® DirEx™
for preperation of PCR-template without extraction

DirEx™		50	250-050	solution

GeneAll® RNA series *for preparation of total RNA*

RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ *plus!*	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD *plus!*	mini	50	312-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Allspin™	mini	50	306-150	spin

GeneAll® *AmpONE™* *for PCR amplification*

Taq DNA polymerase	250 U	501-025	(2.5 U/μℓ)
	500 U	501-050	
	1,000 U	501-100	
α-Taq DNA polymerase	250 U	502-025	(2.5 U/μℓ)
	500 U	502-050	
	1,000 U	502-100	
Pfu DNA polymerase	250 U	503-025	(2.5 U/μℓ)
	500 U	503-050	
	1,000 U	503-100	

Products	Scale	Size	Cat. No.	Type

GeneAll® *AmpONE™* *for PCR amplification*

Hotstart Taq DNA polymerase		250 U	531-025	(2.5 U/μℓ)
		500 U	531-050	
		1,000 U	531-100	
Clean Taq DNA polymerase		250 U	551-025	(2.5 U/μℓ)
		500 U	551-050	
		1,000 U	551-100	
Clean α-Taq DNA polymerase		250 U	552-025	(2.5 U/μℓ)
		500 U	552-050	
		1,000 U	552-100	
Taq Premix	96 tubes	20 μℓ	521-200	lyophilized
		50 μℓ	521-500	
		20 μℓ	526-200	solution
		50 μℓ	526-500	
α-Taq Premix	96 tubes	20 μℓ	522-200	lyophilized
		50 μℓ	522-500	
		20 μℓ	527-200	solution
		50 μℓ	527-500	
HS-Taq Premix	96 tubes	20 μℓ	525-200	solution
		50 μℓ	525-500	
Taq Premix (w/o dye)	96 tubes	20 μℓ	524-200	lyophilized
α-Taq Premix (w/o dye)	96 tubes	20 μℓ	525-200	solution
dNTP mix		500 μℓ	509-020	2.5 mM each
dNTP set (set of dATP, dCTP, dGTP and dTTP)		1 ml x 4 tubes	509-040	100 mM

GeneAll® *AmpMaster™* *for PCR amplification*

Taq Master mix	2x	541-010	0.5 ml x 2 tubes
	2x	541-050	0.5 ml x 10 tubes
α-Taq Master mix	2x	542-010	0.5 ml x 2 tubes
	2x	542-050	0.5 ml x 10 tubes
HS-Taq Master mix	2x	545-010	0.5 ml x 2 tubes
	2x	545-050	0.5 ml x 10 tubes

* Each dNTP is available

Note .

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We thank you for any comment or advice.

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