Ver 4.5

HB1020

Handbook for

- GEL SV
- PCR SV
- CLEANUP SV
- COMBO GP

Expin™ Protocol Handbook

For purification of fragment DNA

GeneAll

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

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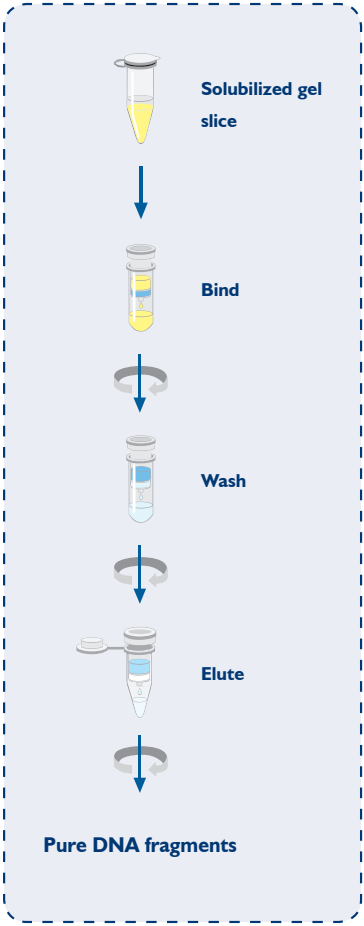
This protocol handbook is included in :

GeneAll® Expin™ Gel SV (102-150, 102-102)
GeneAll® Expin™ PCR SV (103-150, 103-102)
GeneAll® Expin™ CleanUp SV (113-150, 113-102)
GeneAll® Expin™ Combo GP (112-150, 112-102)

Visit www.geneall.com for FAQ, Q&A and more information.

Brief Protocol

Expin™ Gel SV



1. Excise the DNA band of interest
2. Add 3 vol. (μl) Buffer GB to 1 vol. (mg) gel
3. Incubate at 50°C until the gel is completely melted

4. Apply the mixture into mini column
5. Centrifuge for 1 min

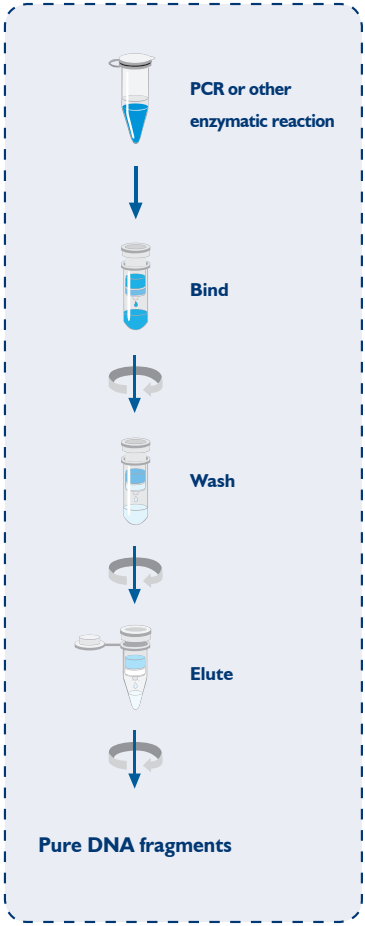
6. Add 700 μl Buffer NW
7. Apply the mixture into mini column
8. Centrifuge for 30 sec

9. Additional centrifuge for 1 min

10. Apply 50 μl Buffer EB
11. Incubate for 1 min at RT
12. Centrifuge for 1 min

Brief Protocol

Expin™ PCR SV



1. Add 5 vol. Buffer PB to 1 vol. sample

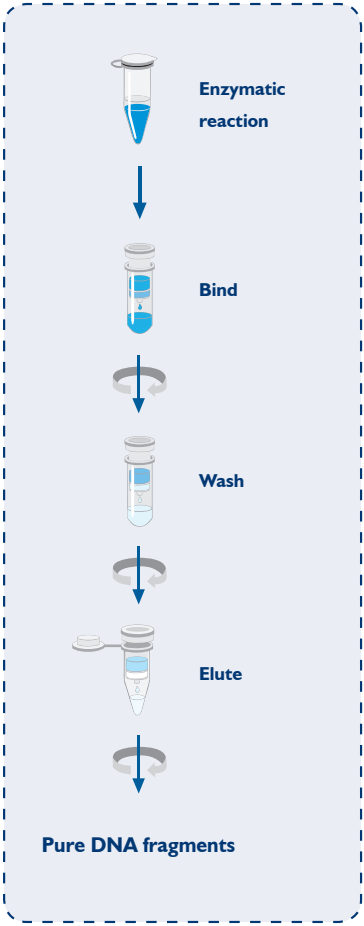
2. Apply the mixture into mini column
3. Centrifuge for 30 sec

4. Add 700 µl Buffer NW
5. Apply the mixture into mini column
6. Centrifuge for 30 sec

7. Additional centrifuge for 1 min

8. Apply 50 µl Buffer EB
9. Incubate for 1 min at RT
10. Centrifuge for 1 min

Expin™ Clean up SV



1. Add 10 vol. Buffer NR to 1 vol. sample

2. Apply the mixture into mini column
3. Centrifuge for 30 sec

4. Add 700 μl Buffer NW
5. Apply the mixture into mini column
6. Centrifuge for 30 sec

7. Additional centrifuge for 1 min

8. Apply 50 μl Buffer EB
9. Incubate for 1 min at RT
10. Centrifuge for 1 min

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PROTOCOLS FOR..

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Kit Contents

	Gel SV		PCR SV	
Cat. No.	**102-150**	**102-102**	**103-150**	**103-102**
Size	**mini**	**mini**	**mini**	**mini**
No. of preparation	50	200	50	200
Column Type D (mini) (with collection tube)	50	200	50	200
Buffer GB	60 ml	120 ml x 2	-	-
Buffer PB	-	-	30 ml	120 ml
Buffer NW (concentrate) * †	12 ml	50 ml	12 ml	50 ml
Buffer EB **	15 ml	30 ml	15 ml	30 ml
Protocol Handbook	1	1	1	1

	CleanUp SV		Combo GP	
Cat. No.	**113-150**	**113-102**	**112-150**	**112-102**
Size	**mini**	**mini**	**mini**	**mini**
No. of preparation	50	200	50	200
Column Type D (mini) (with collection tube)	50	200	50	200
Buffer GB	-	-	60 ml	120 ml x 2
Buffer PB	-	-	30 ml	120 ml
Buffer NR	30 ml	120 ml	-	-
Buffer NW (concentrate) * †	12 ml	50 ml	12 ml	50 ml
Buffer EB **	15 ml	30 ml	15 ml	30 ml
Protocol Handbook	1	1	1	1

** Before first use, add absolute ethanol (ACS grade or better) into Buffer NW as indicated on the bottle*

† *Contains sodium azide as a preservative*

*** 10 mM TrisCl, pH 8.5*

Product Specifications

GeneAll® Expin™	Gel SV	PCR SV	CleanUp SV
Type	spin/mini	spin/mini	spin/mini
Standard sample size	200 mg of gel (400 mg max.)	100 µl of PCR reactions	50 µl of enzyme mixtures
Recovered DNA size	80 bp~10 kb	100 bp~10 kb	40 bp~10 kb
Typical yields	≤85%	≤95%	≤95%
Binding capacity	10 µg	10 µg	10 µg
Preparation time	≥15 min	≥6 min	≥6 min
Maximum loading volume of mini column	750 µl	750 µl	750 µl
Minimum elution volume	30 µl	30 µl	30 µl

Quality Control

All components in GeneAll® Expin™ series are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Storage Conditions

All components of GeneAll® Expin™ series should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight.

During shipment or storage under cool ambient condition, a precipitate can form in Buffer GB, PB or NR. In such a case, heat the bottle to 50°C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery. Expin™ series are guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in the GeneAll® Expin™ series contain irritants which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer GB, PB and NR contain chaotropes, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

GeneAll® Expin™ series is for research use only, not for use in diagnostic procedure.

Product Description

GeneAll® Expin™ series provide reliable and fast methods for the purification of fragment DNA from agarose gel and PCR/enzyme reaction mixtures. Expin™ series consist of;

GeneAll® Expin™ Gel SV (102-150, 102-102)

GeneAll® Expin™ Gel SV kit is designed for a fast and efficient isolation of 80 bp to 10 kb of DNA fragments from standard or low-melting agarose gel in TAE or TBE buffer system. Purified DNA can be directly used in ligation, labelling, sequencing and many other downstream application without further manipulation.

GeneAll® Expin™ PCR SV (103-150, 103-102)

GeneAll® Expin™ PCR SV kit provides a simple and rapid method to purify PCR products or other enzymatic reactions in just 6 minutes. Up to 10 μg of pure DNA (100 bp~10 kb) can be obtained, and this purified DNA can be directly used in cloning, sequencing and many other application. Expin™ PCR SV procedures remove the DNA fragment smaller than 100 bp, resulting in removal of primers and primer dimers in PCR products.

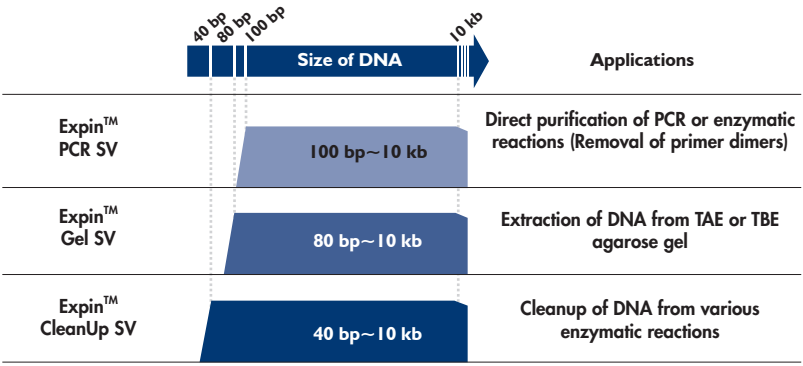
GeneAll® Expin™ CleanUp SV (113-150, 113-102)

GeneAll® Expin™ CleanUp SV kit provides a simple and rapid method to purify 40 bp~10 kb DNA from enzymatic reactions in just 6 minutes. Up to 10 μg of pure DNA which is at least 40 bp but less than 10 kb in length can be obtained using this kit and the purified DNA can be directly used for sequencing, cloning and other routine applications without further manipulation.

GeneAll® Expin™ Combo GP (112-150, 112-102)

The combination set of GeneAll® Expin™ Gel SV and Expin™ PCR SV.

- Applicable range in length of DNA



- General Considerations

GeneAll® Expin™ series takes advantage of silica membrane and spin/vacuum column technology to recover DNA fragments. Under high salt conditions, DNA binds to silica membrane and impurities pass through membrane into a collection tube. The membranes are washed with an ethanol-containing buffer to remove any traces of proteins, salts, remnants, of agarose and other enzymatic reaction components. Finally pure DNA is released into a clean collection tube with water or low ionic strength buffer.

- Binding

The basic principle which DNA binds to silica membrane is identical in all GeneAll® Expin™ series. Binding buffers (Buffer GB, PB and NR) make the optimal binding condition in each specific applications. Buffer GB in Expin™ Gel SV kit is composed to dissolve standard agarose gel as well as low melt agarose gel in addition to adjusting a binding condition. Usually low melt agarose gel results in a better recovery yield. DNA binds to silica membrane at lower pH than pH 7.5. The components and pH of starting sample can alter the pH of the mixture with binding buffer, especially in extraction of DNA from agarose gel. Buffer GB contains pH indicator in order to check this alteration of binding condition. If the color (yellow) of binding mixture turns to brown or purple after addition of Buffer GB, it means that the pH of binding mixtures is higher than the optimal, and it can be easily adjusted with small volume of sodium acetate before proceeding with the protocol. The indicator dye is completely removed during subsequent washing steps and does not interfere the downstream applications.

- Washing

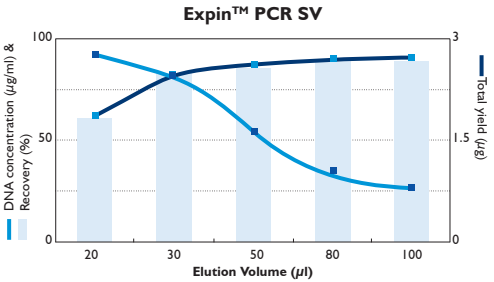
Any unwanted oligos and impurities, such as salts, proteins, nucleotides, agarose, dyes and detergents will not be bound but be passed through the silica membrane. A minute impurities, such as salts, are quantitatively washed away with Buffer NW which contains ethanol. The quality of DNA can be slightly increased with the repeat of washing. Any residual ethanol should be removed completely with an additional centrifugation because the residual ethanol in eluate may interfere some subsequent applications.

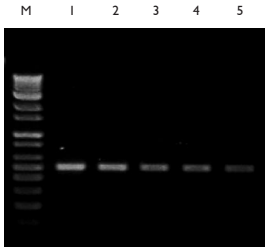
- Elution

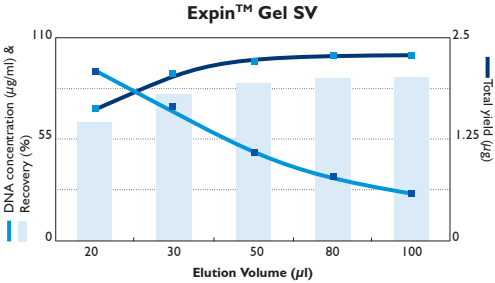
DNA is released under the condition of low salts and neutral or weakly alkaline pH (7.0<pH<9.5). Although Buffer EB (10 mM TrisCl, pH 8.5), TE, or distilled water can be used for elution, it should be considered that EDTA in buffer TE may interfere the subsequent reactions and low pH (<7.0) of distilled water can reduce DNA recovery. Because water does not have any buffering agents the eluate in water should be stored under -20°C not to degrade.

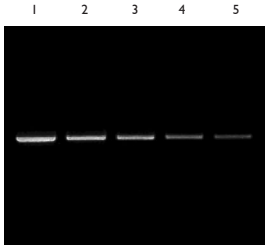
The minimum elution volume is 30 μl and lower volume will decrease the yield significantly. It is important for optimal elution to apply the elution buffer to the center of the membrane, because the membrane should be covered completely by eluent for an optimal recovery. Up to 200 μl of elution buffer can be applied and it results in low concentration of DNA. Higher concentrated DNA will be obtained with lower elution volume, and maximum yield can be obtained by larger elution volume. The yield with large fragments (>5 kb) can be increased slightly by using pre-warmed (70°C) elution buffer. Incubation for 1 minute after addition of eluent may increase the efficiency of elution.

- Correlation between the elution volume and the recovery







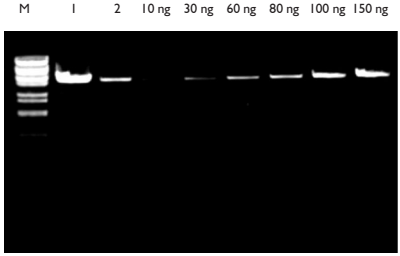


Correlation between the elution volume and the recover rates. Upper panel; Expin™ PCR SV kit. Lower panel; Expin™ Gel SV kit. 3 μg of 494 bp PCR products were purified and eluted with the indicated volumes of Buffer EB. Elution volume lower than 30 μl causes significant loss of DNA. 1/10 volume of eluate purified with Expin™ Gel SV kit was resolved on 1% agarose gel; lower right. (1/20 for Expin™ PCR SV kit, upper right)

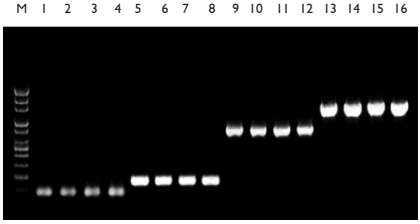
	Recovery rates (%)		
DNA size (bp)	Gel SV	PCR SV	CleanUp SV
60	39	0	63
120	71	78	80
200	76	83	84
800	84	94	94
1800	82	91	93
4300	78	85	88
8700	73	76	79

Average recovery rates of Expin™ series with various sizes of DNA. 3 µg of starting sample was purified and eluted with 50 µl of Buffer EB. Optional steps were not performed and mini columns were incubated for 1 min after addition of Buffer EB.

- Electrophoresis analysis



*Quantities of extracted 4.5 kb DNA fragment corresponding to 1/5 of the DNA obtained by purification from 0.5 µg starting DNA with a recovery of 90%. Sample were run on 1% TAE agarose gel. M; Lambda-BstP1 marker Lane1; Total amount before extraction (0.5 µg) Lane2; 1/5 amount after extraction [90 ng compared to known amount (10~150 ng) DNA] * Total obtained amount of DNA=90 x 5=450 ng approximately (90%)*



PCR products of several sizes (70, 176, 757 and 1487 bp from left to right) were purified using Expin™ CleanUp SV kit. The band intensity of 1/5 amount of 20 µl PCR products (lane 1, 5, 9, 13) was almost identical with that of 1/5 amount of 50 µl eluate. (Lane 2~4, 6~8, 10~12 and 14~16, tripli-cate) M; 1 kb ladder marker

Expin™ Gel SV Protocol

Before experiment

* *Before first use, add absolute ethanol (ACS grade or better) to Buffer NW as indicated on the bottle.*
* *All centrifugation should be carried out at 10,000 x g above (>12,000 rpm) at room temperature in a microcentrifuge.*
* *Prepare water bath or heating block to 50°C.*
* *All solutions should be equilibrated at room temperature before procedures.*
* *For large fragments (>5 kb), pre-warm Buffer EB at 70°C.*
* *If a precipitate is formed in Buffer GB, heat at 50°C to dissolve before use.*

Spin/Vacuum Methods for Gel Extraction

When using this kit, one of the two methods can be chosen to purify DNA. DNA fragment can be purified using centrifugation to pull the mixture through the mini column. Alternatively, vacuum can be used to force the mixture out through the column.

A. Centrifugation Protocol

1. **Excise the DNA band of interest using a ethanol-cleaned razor blade or scalpel on a transilluminator.**
   Minimize gel volume by cutting the gel slice as small as possible.
   Use of long wave length transilluminator and short handling time will lead to better quality of DNA. It can be critical in some experiments, such as ligation.

2. **Weigh the gel slice in a 1.5 ml microcentrifuge tube. Add 3 volumes (μl) of Buffer GB to 1 volume (mg) of gel.**
   For example, add 300 μl of Buffer GB to each 100 mg of agarose gel slice. For >1.5% agarose gel, add 5 volumes of Buffer GB.

3. **Incubate at 50°C until the agarose gel is completely melted (5~10 min).**
   To help the efficient dissolving of gel, vortex the tube every 2~3 min during the incubation.

4. **After the slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer GB).**
   If the color of the mixture becomes brown or purple, add 10 μl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. Adjusting of pH is not needed when the change of color is due to the ingredient of loading dye (eg. bromophenol blue, xylene cyanol).

5. ***(Optional:)* Add 1 gel volume of isopropanol to the sample and vortex to mix.**
   For 100 mg of gel volume, add 100 μl of isopropanol.
   Do NOT centrifuge at this step.
   This step is required to increase the recover yields of DNA fragments <200 bp or >5 kb. For the DNA fragments between 200 bp and 5 kb, it has little effect on the recovery.

6. **Transfer the mixture to a Column Type D (mini). Centrifuge for 1 min at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column into the collection tube.**
   If the mixture volume is larger than 700 μl, apply the mixture twice; apply 700 μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat the step again until all of the mixture has been applied to the mini column.

7. ***(Optional:)* Apply 500 μl of Buffer GB to the mini column. Centrifuge for 30 sec at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column into the collection tube.**
   This step is for further complete removal of any traces of agarose and required only for direct use of purified DNA for very sensitive applications, such as in vitro transcription. Usually this step can be skipped for automatic sequencing or ligation.

8. Add 700 μl of Buffer NW to the mini column. Centrifuge for 30 sec at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column into the collection tube.

If the purified DNA will be used for salt sensitive applications, let the mini column stand for 5 min after addition of Buffer NW, making some amount of wash buffer flow through the column by gravity before centrifugation.

9. Centrifuge for an additional 1 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.

If residual ethanol remains in the mini column, centrifuge again for an additional 1 min at full speed before transferring to a new 1.5 ml microcentrifuge tube. Residual ethanol from Buffer NW can inhibit subsequent enzymatic reaction.

10. Apply 50 μl of Buffer EB or ddH_2O to the center of the membrane in the mini column, let stand for 1 min and centrifuge for 1 min at 10,000 x g above (>12,000 rpm).

Ensure that the Buffer EB or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

To obtain more concentrated DNA solution, apply 30 μl of elution buffer, but the volume lower than 30 μl will decrease the yield significantly. Up to 200 μl of elution buffer can be applied to mini column, and it will reduce the concentration of DNA.

For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.

For long-term storage, eluting in Buffer EB (10 mM TrisCl, pH 8.5) or TE, pH 8.0 and storing under -20°C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.

B. Vacuum Protocol

The vacuum pressure should be in the range of the list below. Lower vacuum pressure may reduce DNA yield and purity.
Most commercial vacuum manifold with luer connectors can be used with this protocol.

15~18 in Hg	380~460 mbar
285~345 mm Hg	5.5~6.5 Psi

1. **Excise the DNA band of interest using a ethanol-cleaned razor blade or scalpel on a transilluminator.**
   Minimize gel volume by cutting the gel slice as small as possible.
   Use of long wave length transilluminator and short handling time will lead to better quality of DNA. It can be critical in some experiments, such as ligation.

2. **Weigh the gel slice in a microcentrifuge tube. Add 3 volumes (μl) of Buffer GB to 1 volume (mg) of gel.**
   For example, add 300 μl of Buffer GB to each 100 mg of agarose gel slice. For >1.5% agarose gel, add 5 volumes of Buffer GB.

3. **Incubate at 50°C until the agarose gel is completely melted (5~10 min).**
   To help the efficient dissolving of gel, vortex the tube every 2~3 min during the incubation.

4. **After the slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer GB).**
   If the color of the mixture becomes brown or purple, add 10 μl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. Adjusting of pH is not needed when the change of color is due to the ingredient of loading dye (eg. bromophenol blue, xylene cyanol)

5. ***(Optional:)*** **Add 1 gel volume of isopropanol to the sample and vortex to mix.**
   For 100 mg of gel volume, add 100 µl of isopropanol. DO NOT centrifuge at this step.
   This step is required to increase the recover yields of DNA fragments <200 bp or >5 kb. For the DNA fragments between 200 bp and 5 kb, it has little effect on the recovery.

6. **Attach a Column Type D (mini) to a port of the vacuum manifold tightly.**
   Most commercial vacuum manifold with luer connectors can be adopted to this protocol.

7. **Transfer the mixture to the mini column by pipetting. Switch on vacuum source to draw the solution through the mini column. When all liquid has been pulled through the mini column, release the vacuum. Repeat this step until all mixture have applied to the mini column.**

8. ***(Optional:)*** **Apply 500 µl of Buffer GB to the mini column and switch on vacuum source. When all liquid has been pulled through the mini column, release the vacuum.**
   This step is for further complete removal of any traces of agarose and required only for direct use of purified DNA for very sensitive applications, such as in vitro transcription. Usually this step can be skipped for automatic sequencing.

9. **Apply 700 µl of Buffer NW and switch on vacuum source. When all liquid has been pulled through the mini column, release the vacuum. Transfer the mini column to a empty collection tube (provided).**
   If the purified DNA will be used for salt sensitive applications, let the mini column stand for 5 min after addition of Buffer NW before applying vacuum.

10. **Centrifuge for an additional 1 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.**
    If the mini column has Buffer NW associated with it, centrifuge again for additional 1 min at full speed before transferring to a new 1.5 ml microcentrifuge tube. Residual ethanol from Buffer NW can inhibit subsequent enzymatic reaction.

11. Apply 50 μl of Buffer EB or ddH_2O to the center of the membrane in the mini column, let stand for 1 min and centrifuge for 1 min at 10,000 x g above (>12,000 rpm).

Ensure that the Buffer EB or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

To obtain more concentrated DNA solution, apply 30 μl of elution buffer, but the volume lower than 30 μl will decrease the yield significantly. Up to 200 μl of elution buffer can be applied to mini column, and it will reduce the concentration of DNA.

For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.

For long-term storage, eluting in Buffer EB (10 mM TrisCl, pH 8.5) or TE, pH 8.0 and storing under -20°C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.

Expin™ PCR SV Protocol

Before experiment

* *Before first use, add absolute ethanol (ACS grade or better) to Buffer NW as indicated on the bottle.*
* *All centrifugation should be carried out at 10,000 x g above (>12,000 rpm) at room temperature in a microcentrifuge.*
* *All solutions should be equilibrated at room temperature before procedures.*
* *For large fragments (>5 kb), pre-warm Buffer EB at 70°C.*

Spin/Vacuum Methods for PCR Purification

When using this kit, one of the two methods can be chosen to purify DNA. DNA fragment can be purified using centrifugation to pull the mixture through the mini column. Alternatively, vacuum can be used to force the mixture through the column.

A. Centrifugation Protocol

1. **Add 5 volumes of Buffer PB to 1 volume of the sample and mix. Transfer the mixture to a Column Type D (mini).**
   For 100 μl reaction, add 500 μl of Buffer PB. It is not necessary to remove mineral oil.

2. **Centrifuge for 30 sec at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column back into the same tube.**

3. **Apply 700 μl of Buffer NW and centrifuge for 30 sec at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column back into the collection tube.**

4. **Centrifuge for an additional 1 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.**

   If the mini column has Buffer NW associated with it, centrifuge again for additional 1 min at full speed before transferring to the new 1.5 ml microcentrifuge tube. Residual ethanol from Buffer NW can inhibit subsequent enzymatic reaction.

5. **Apply 50 μl of Buffer EB or ddH_2O to the center of the membrane in the mini column, let stand for 1 min and centrifuge for 1 min at 10,000 x g above (>12,000 rpm).**

   Ensure that the Buffer EB or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

   To obtain more concentrated DNA solution, apply 30 μl of elution buffer, but the volume lower than 30 μl will decrease the yield significantly. Up to 200 μl of elution buffer can be applied to mini column, and it will reduce the concentration of DNA.

   For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.

   For long-term storage, eluting in Buffer EB (10 mM TrisCl, pH 8.5) or TE, pH 8.0 and storing under -20°C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.

B. Vacuum Protocol

The vacuum pressure should be in the range of the list below. Lower vacuum pressure may reduce DNA yield and purity.
Most commercial vacuum manifold with luer connectors can be used with this protocol.

15~18 in Hg	380~460 mbar
285~345 mm Hg	5.5~6.5 Psi

1. **Attach a Column Type D (mini) to a port of the vacuum manifold tightly.**

2. **Add 5 volumes of Buffer PB to 1 volume of the sample and mix. Transfer the mixture to the mini column by pipetting.**
   For 100 μl reaction, add 500 μl of Buffer PB.
   It is not necessary to remove mineral oil.

3. **Switch on vacuum source to draw the solution through the mini column. When all liquid has been pulled through the mini column, release the vacuum.**

4. **Apply 700 μl of Buffer NW and switch on vacuum source. When all liquid has been pulled through the mini column, release the vacuum. Transfer the mini column to a collection tube (provided).**

5. **Centrifuge for 1 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.**
   If the mini column has Buffer NW associated with it, centrifuge again for additional 1 min at full speed before transferring to the new 1.5 ml microcentrifuge tube. Residual ethanol from Buffer NW can inhibit subsequent enzymatic reaction.

6. Apply 50 μl of Buffer EB or ddH_2O to the center of the membrane in the mini column, let stand for 1 min and centrifuge for 1 min at 10,000 x g above (>12,000 rpm).

Ensure that the Buffer EB or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

To obtain more concentrated DNA solution, apply 30 μl of elution buffer, but the volume lower than 30 μl will decrease the yield significantly. Up to 200 μl of elution buffer can be applied to mini column, and it will reduce the concentration of DNA.

For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.

For long-term storage, eluting in Buffer EB (10 mM TrisCl, pH 8.5) or TE, pH 8.0 and storing under -20°C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.

Expin™ CleanUp SV Protocol

Before experiment

* *Before first use, add absolute ethanol (ACS grade or better) to Buffer NW as indicated on the bottle.*
* *All centrifugation should be carried out at 10,000 x g above (>12,000 rpm) at room temperature in a microcentrifuge.*
* *All solutions should be equilibrated at room temperature before procedures.*
* *For large fragments (>5 kb), pre-warm Buffer EB at 70°C.*

Spin/Vacuum Methods for DNA Clean-up

When using this kit, one of the two methods can be chosen to purify DNA. DNA fragment can be purified using centrifugation to pull the mixture through the mini column. Alternatively, vacuum can be used to force the mixture through the column.

A. Centrifugation Protocol

1. **Add 10 volumes of Buffer NR to 1 volume of the sample and mix. Transfer the mixture to a Column Type D (mini).**
   For 50 μl reaction, add 500 μl of Buffer NR.
   If the length of DNA is longer than 100 bp, add 5 volumes of Buffer NR.

2. **Centrifuge for 30 sec at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column back into the collection tube.**

3. **Apply 700 μl of Buffer NW and centrifuge for 30 sec at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column back into the collection tube.**

4. **Centrifuge for an additional 1 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.**

   If the mini column has Buffer NW associated with it, centrifuge again for additional 1 min at full speed before transferring to the new 1.5 ml microcentrifuge tube. Residual ethanol from Buffer NW can inhibit subsequent enzymatic reaction.

5. **Apply 50 μl of Buffer EB or ddH_2O to the center of the membrane in the mini column, let stand for 1 min and centrifuge for 1 min at 10,000 x g above (>12,000 rpm).**

   Ensure that the Buffer EB or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

   To obtain more concentrated DNA solution, apply 30 μl of elution buffer, but the volume lower than 30 μl will decrease the yield significantly. Up to 200 μl of elution buffer can be applied to mini column, and it will reduce the concentration of DNA.

   For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.

   For long-term storage, eluting in Buffer EB (10 mM TrisCl, pH 8.5) or TE, pH 8.0 and storing under -20°C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.

B. Vacuum Protocol

The vacuum pressure should be in the range of the list below. Lower vacuum pressure may reduce DNA yield and purity.

Most commercial vacuum manifold with luer connectors can be used with this protocol.

15~18 in Hg	380~460 mbar
285~345 mm Hg	5.5~6.5 Psi

1. **Attach a Column Type D (mini) to a port of the vacuum manifold tightly.**

2. **Add 10 volumes of Buffer NR to 1 volume of the sample and mix. Transfer the mixture to the mini column by pipetting.**
   For 50 μl reaction, add 500 μl of Buffer NR.
   If the length of DNA is longer than 100 bp, add 5 volumes of Buffer NR.

3. **Switch on vacuum source to draw the solution through the mini column. When all liquid has been pulled through the mini column, release the vacuum.**

4. **Apply 700 μl of Buffer NW and switch on vacuum source. When all liquid has been pulled through the mini column, release the vacuum. Transfer the mini column to a collection tube (provided).**

5. **Centrifuge for an additional 1 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.**
   If the mini column has Buffer NW associated with it, centrifuge again for additional 1 min at full speed before transferring to the new 1.5 ml microcentrifuge tube. Residual ethanol from Buffer NW can inhibit subsequent enzymatic reaction.

6. Apply 50 μl of Buffer EB or ddH_2O to the center of the membrane in the mini column, let stand for 1 min and centrifuge for 1 min at 10,000 x g above (>12,000 rpm).

Ensure that the Buffer EB or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

To obtain more concentrated DNA solution, apply 30 μl of elution buffer, but the volume lower than 30 μl will decrease the yield significantly. Up to 200 μl of elution buffer can be applied to mini column, and it will reduce the concentration of DNA.

For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.

For long-term storage, eluting in Buffer EB (10 mM TrisCl, pH 8.5) or TE, pH 8.0 and storing under -20°C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	**[Gel SV] Incompletly solubilized gel**	The sliced agarose gel should be completely dissolved without any particles. To assist the complete solubilization, mix the tube by vortexing every 2~3 min during incubation or increase the incubation time. To use low melt agarose usually results in better recovery.
	[Gel SV] Too high pH of binding mixture	At high pH, the binding of DNA to silica membrane will be significantly reduced. The dye included in Buffer GB indicates the pH of mixture as color change from yellow at optimal pH to brown or purple at abnormally higher pH. If the color of mixture has turned to brown or purple, add 10 μl of 3 M sodium acetate, (pH 5.0) to the sample and mix. The color of mixture will turn to yellow indicating the correct pH for DNA binding.
	Improper elution buffer	As user's requirement, elution buffer other than Buffer EB can be used. The condition of optimal elution is low salt concentration with alkaline pH (7.0<pH<9.5). When water or other buffer was used as eluent, ensure that conditions.
	Elution buffer incorrectly dispensed	Ensure that elution buffer dispensed to the center of membrane. Incorrectly dispensed elution buffer causes inappropriate contact with membrane, followed by poor DNA recovery.
Ligation failure	**[Gel SV] Too long or strong exposure to UV on transilluminator**	UV destroys the DNA ends. Use UV of long wave length and make the handling time as short as possible when excising the gel slice.

Facts	Possible Causes	Suggestions
Clogged membrane	**[Gel SV] Incompletly solubilized gel**	See the section 'Incompletely solubilized gel' in the Facts "Low or no recovery"
	[Gel SV] >1.5% agarose gel is used	For >1.5% agarose gel, 5 volumes of Buffer GB to 1 volume of gel slice should be added. For 100 mg of agarose gel, add 500 μl of Buffer GB. If the mini column is clogged, transfer the mixture from the mini column to a 1.5 ml microcentrifuge tube, add 1 volume of Buffer GB to mixture volume. Incubate for 5 min at 50°C, proceed again to binding steps.
Enzymatic reaction is not performed well with the purified DNA	**Residual ethanol from Buffer NW remains in eluate**	It is essential to remove any residual ethanol included in Buffer NW from column membrane. Centrifuge again for complete removal of ethanol.
	Too high salt concentration in eluate	Incubate for 5 min after addition of Buffer NW at washing steps.
	Eluate contains denatured ssDNA	For reannealing of ssDNA to dsDNA, incubate ssDNA at 95°C for 2 min, and then allow to cool slowly to room temperature.
DNA floats out while loading on agarose gel	**Residual ethanol from Buffer NW remains in eluate**	It is essential to remove any residual ethanol included in Buffer NW from column membrane. Centrifuge again for complete removal of ethanol.
Non-specific band appears after purification	**DNA denatured**	Renature the DNA by warming up to 95°C for 1 min and let cool slowly to room temperature.

Ordering Information

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

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	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	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™* *for preparation of transfection-grade 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
Stool DNA mini	mini	50	115-150	spin
Stool-Bead DNA mini	mini	50	115-151	spin
Viral DNA / RNA	mini	50	128-150	spin
FFPE Tissue DNA	mini	50	138-150	spin
		250	138-152	

GeneAll® *GenEx™* *for isolation of total DNA without spin column*

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™* series *for preperation of PCR-template without extraction*				
DirEx™		100	250-101	solution
DirEx™ *Fast*-Tissue		96 T	260-011	solution
DirEx™ *Fast*-Cultured cell		96 T	260-021	solution
DirEx™ *Fast*-Whole blood		96 T	260-031	solution
DirEx™ *Fast*-Blood stain		96 T	260-041	solution
DirEx™ *Fast*-Hair		96 T	260-051	solution
DirEx™ *Fast*-Buccal swab		96 T	260-061	solution
DirEx™ *Fast*-Cigarette		96 T	260-071	solution
GeneAll® RNA series *for preperation 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™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD *plus!*	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed / Fruit	mini	50	317-150	spin
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351-001	solution

Products	Scale	Size	Cat. No.	Type
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	
Taq Premix	20 μl x 96 tubes		526-200	solution
	50 μl x 96 tubes		526-500	
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix	0.5 ml x 2 tubes		541-010	solution
	0.5 ml x 10 tubes		541-050	solution
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase		10,000 U	601-100	solution
RT Master mix	0.5 ml x 2 tubes		601-710	solution
One-step RT-PCR Master mix	0.5 ml x 2 tubes		602-110	solution
One-step RT-PCR Premix	20 μl x 96 tubes		602-102	solution
GeneAll® RealAmp™ *for qPCR amplification*				
SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	

Products	Scale	Size	Cat. No.	Type

GeneAll® Protein series

ProtinEx™ Animal cell / tissue	100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001	solution

GeneAll® STEADi™ *for automatic nucleic acid purification*

12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell / Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Total RNA	96	404-304	kit
Viral DNA / RNA	96	405-322	kit
Genomic DNA Plant	96	407-117	kit
Soil DNA	96	408-114	kit

GeneAll® GENTi™ 32 *Ultimately flexible automatic extraction system*

Automatic extraction equipment		GTI032	system
Genomic DNA	48	901-048	tube
	96	901-096	plate
Viral DNA / RNA	48	902-048	tube
	96	902-096	plate
Whole Blood Genomic DNA	48	903-048	tube
	96	903-096	plate

Products	Scale	Size	Cat. No.	Type

GeneAll® GENTi™ 32 ADVANCED *Ultimately flexible automatic extraction system*

Automatic extraction equipment		GTI032A	system
Genomic DNA	48	901-048A	tube
	96	901-096A	plate
Viral DNA / RNA	48	902-048A	tube
	96	902-096A	plate
Plant DNA / RNA	48	904-048A	tube
	96	904-096A	plate
Blood DNA	48	903-048A	tube
	96	903-096A	plate

NOTE

GeneAll

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