User Manual

GenElute™-E Single Spin Cell Culture DNA Kit

For Purification of Genomic DNA from Cell Culture Samples

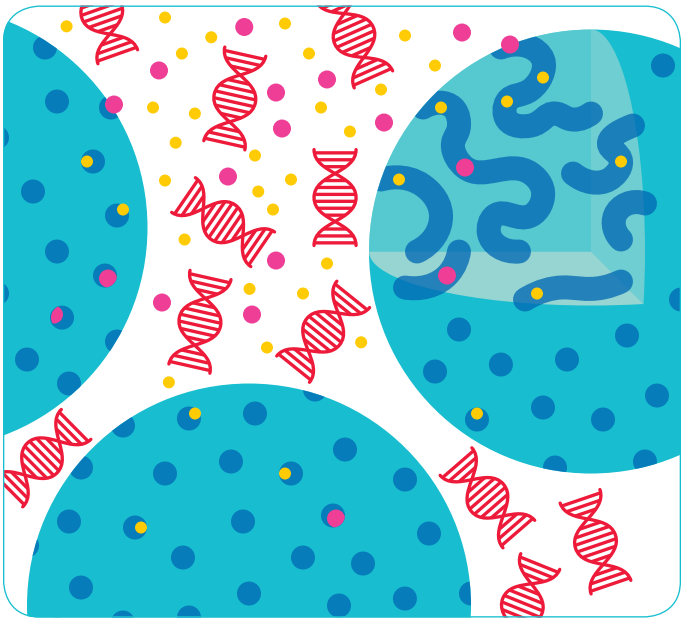
EC400

What's Inside:

For research use only.

Introduction

GenElute™-E Single Spin Kit is a nucleic acid purification system that eliminates the need for high salt binding and ethanol wash steps, yielding DNA and RNA preparations with fewer impurities for more robust results. GenElute™-E DNA and RNA purification kits employ a **negative chromatography** method dependent on size exclusion to separate large DNA and RNA nucleic acid molecules from smaller protein, lipid, and ionic components in cell, tissue, blood, and other samples.



Using negative chromatography, Single Spin columns efficiently absorb and retain sample contaminants while allowing nucleic acids to flow through the column, reducing the number of steps and plastic materials required for purification. The key is the novel lysis that allows negative chromatography to be used for high quality nucleic acid purification.

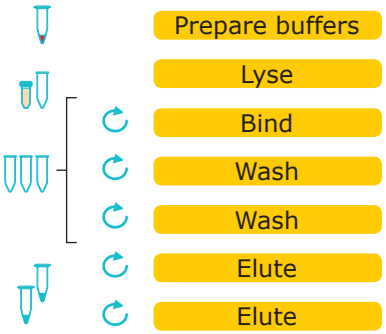
Three key advantages over silica:

- Simplified workflow
- Superior performance
- Waste reduction

A Simplified Workflow

Purification in one spin, eliminating all wash steps and reducing tube handling for more efficient, safer sample processing.

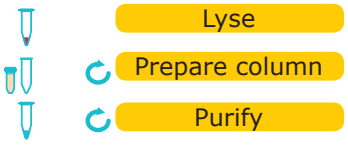
Silica Procedure



Multiple spins
and tube transfers

Lysis
45 minutes to overnight
Purification
45 minutes hands-on time
6 centrifugation steps

GenElute™-E Single Spin Technology



- ✓ Reduced time
- ✓ Reduced handling
- ✓ Reduced waste

Lysis
10 to 40 minutes
Purification
3 minutes hands-on time
1 centrifugation step

Reduced Waste for a Better Environment

With fewer plastic tubes and no hazardous liquids, GenElute™-E DNA and RNA purification kits provide an eco-friendly alternative to silica-based purification.

GenElute™-E purification kits greatly reduce the amounts of plastic-based components packaged with each kit and consumed while executing protocols in the lab. All tedious binding and washing steps associated with silica-based procedures are omitted, with no use of hazardous materials such as chaotropic salts or organic solvents that require special disposal. Plastic waste is reduced by 55% compared to a common silica kits, resulting in disposal cost savings and reduced environmental impact.

GenElute™-E Single Spin nucleic acid purification kits provide easier workflows for DNA and RNA isolation, better nucleic acid quality with fewer impurities, and reduced plastic and hazardous waste disposal compared to silica bind-wash-elute spin prep kits.

GenElute™-E Single Spin Purification supports:

- Significantly reduced plastic waste
- No hazardous bind and wash steps
- Responsible and sustainable nucleic acid purification
- Disposal cost savings

GenElute™-E kits

Supplier X

Specifications

Sample Input	Up to 1 x 10^6 cultured cells
Sample Type	Cultured human and animal cells
Sample Condition	Fresh, frozen
Required time after lysis	2 minutes
Purified Nucleic Acid	DNA > 200 bp
Elution Volume	90-110 µl
The purified genomic DNA is ready for immediate use in downstream applications:	• Restriction digestions • PCR and qPCR • Southern blots • Sequencing reactions

Intended Use

For single-step purification of genomic DNA from cultured cells. This protocol has been developed for up to 1 x 10^6 human or animal cells.

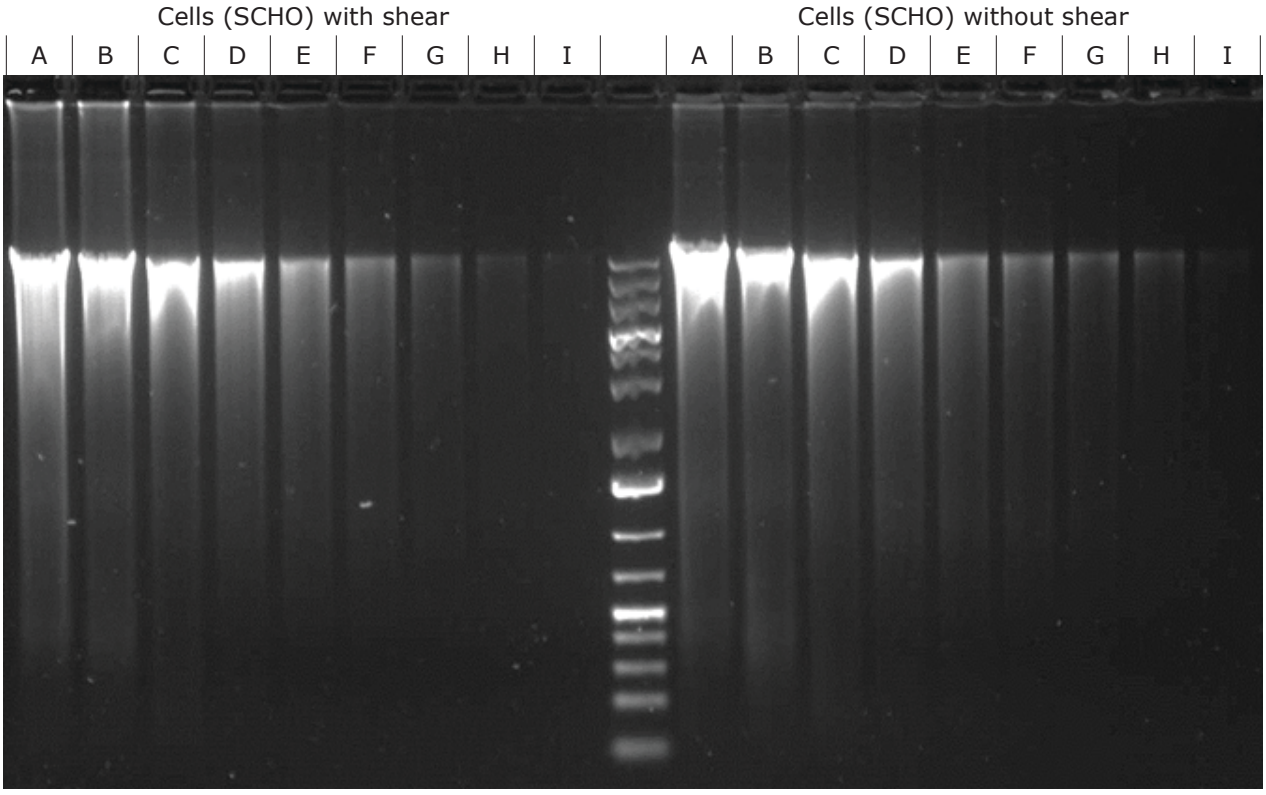
Typical Results

Table 1.

Sample Type	Typical Yield (µg/10mg)
K562	5-20
HEK 293F	10-15
CHO	10-20
NIH 3T3	5-10
Jurkat	10-20

Due to sample variability, results may vary.

Figure 1.



Gel Electrophoresis of gDNA isolation from SCHO cells with and without shearing for different amounts of cells where A) 4 x 10^6 cells, B) 2 x 10^6 cells, C) 1 x 10^6 cells, D) 5 x 10^5 cells, E) 2.5 x 10^5 cells, F) 1.25 x 10^5 cells, G) 6.25 x 10^4 cells, H) 3.13 x 10^4 cells, and I) 1.56 x 10^4 cells using GenElute™-E Single Spin Cell Culture DNA Kit.

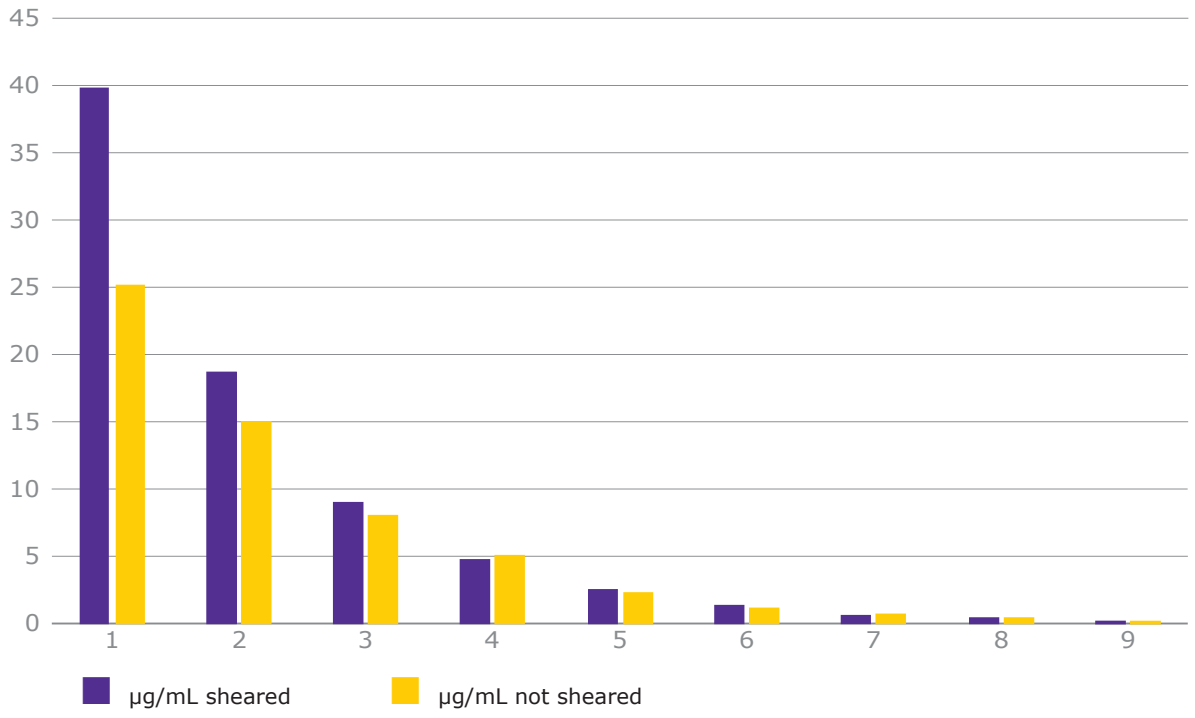
Table 2.

	Average Concentration (ng/µl) sheared	Average Concentration (ng/µl) not sheared		Average Concentration (ng/µl) sheared	Average Concentration (ng/µl) not sheared
4 x 10^6 cells	39.77	25.15	1.25 x 10^5 cells	1.36	1.16
2 x 10^6 cells	18.73	15.03	6.25 x 10^4 cells	0.67	0.71
1 x 10^6 cells	9.03	8.11	3.13 x 10^4 cells	0.30	0.32
5 x 10^5 cells	4.78	5.12	1.56 x 10^4 cells	0.14	0.13
2.5 x 10^5 cells	2.53	2.33			

Average spectrophotometric results of gDNA isolation from SCHO cells with and without shearing for different amounts of cells using GenElute™-E Single Spin Cell Culture DNA Kit. Concentration comparison of sheared and not sheared cells is shown in a graph as Figure 2.

Figure 2.

SCHO Cell Dilution Series



Storage and Stability

Kit Storage

Store SmartLyse™ C Protease (P) and RNase A Cell (R) at 2-8 ºC. The remaining components should be stored at room temperature. Use the kit within 12 months of receipt.

Sample Storage and Variability

Nucleic acid degrades over time, potentially leading to reduced fragment length and overall yield. Therefore, it is best if samples are acquired from fresh material. Be aware that the DNA yield to be expected is cell type dependent.

Disposal

GenElute™-E kits adhere to the principles of "SMASH Packaging", our plan that drives improvement in the sustainability of our packaging through less packaging, more sustainable materials and easier recycling.

The box and insert material comes from sustainably managed forests and/or more than 70% of recycled content. The kit componet bags are composed of starch-based, compostable material. Please recycle.

Kit components exposed to samples should be disposed of with biological waste. Other kit materials should be disposed of according to all applicable international, federal, state, and local regulations.

Materials and Equipment Needed

Kit Contents

- Cell Lysis Buffer (LB)
- SmartLyse™ C Protease (P)
- Clearing Solution C (CS)
- RNase A Cell (R)
- 1x Tris Buffer (T)
- Spin Columns

Not Supplied in Kit

- Microcentrifuge with rotor for 1.5 mL and 2 mL reaction tubes.

  Important: Set centrifuge to relative centrifugal force, rcf (x g). If needed, calculate equivalent rpm by the formula:

  rpm = 1,000 x √(g/(1.12 x r)),
  where r = radius of rotor in mm
  and g is the required g-force.

- Thermal shaker with agitation, capable of heating to 60 °C and 80 °C.

  Alternative: Heating Block or heat chamber.

- Vortex device.
- Pipets for 10 µL and 200 µL scales, corresponding pipet tips.
- One reaction tube (1.5 mL) per sample for the lysis step.
- One reusable reaction tube (2 mL) per sample for column preparation.
- One reaction tube (1.5 mL) per sample for collection of the purified DNA.
- GenElute™-E Single Spin Cap Puncher (optional)

Preparation before starting

- Heat the thermal shaker or heating block/chamber to 60 °C.
- Set the microcentrifuge to 2,000 x g (cell pelleting) or 1,000 x g (column preparation).

Standard Protocol

Lysis

1. Harvest cells (up to 1x10^6 cells) by centrifugation at 2,000 x g for 1 minute in a 1.5 mL reaction tube and remove supernatant carefully. Discard supernatant.

   Important: Make sure that the cell pellet is retained during this step.

2. For each sample, add 55 µL Cell Lysis Buffer LB and 25 µL SmartLyse™ C Protease Mix P. If working with more than two samples, prepare a Lysis Master Mix with 10% excess volume for the number of samples (see table).

   Lysis Master Mix

Number of samples	1	6 (+10%)	12 (+10%)
Cell Lysis Buffer LB	55 µL	363 µL	726 µL
SmartLyse™ C Protease P	25 µL	165 µL	330 µL
Final Volume	80 µL	528 µL	1,056 µL

   Add 80 µL of the Lysis Master Mix to a 2 mL reaction tube.

3. Pulse-vortex to resuspend cell pellet.

4. Place the reaction tube(s) in the thermal shaker and incubate at 60 °C for 10 minutes with maximum agitation.

   If using Heating Block or heat chamber, vortex halfway through incubation time to re-suspend, and return to incubation.

5. After having performed lysis, add 10 µL Clearing Solution C CS and 1 µL RNase ® to the sample. Vortex vigorously with four pulses of 10 seconds each. The sample becomes cloudy.

6. Incubate for 2 minutes at room temperature.

7. Centrifuge for 2 minutes at maximum speed.

Column Preparation

8. Vortex the Spin Column briefly and place into a 2 mL reaction tube. Let stand for 5 to 10 minutes.

9. Loosen the screw cap of the Spin Column and snap off bottom closure of the column. The screw cap must stay loosened half a turn to avoid generation of a vacuum. Place the Spin Column back into the 2 mL reaction tube.

10. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the column buffer.

11. Place the prepared Spin Column into a new 1.5 mL reaction tube for collection of the purified DNA and place back into the rack.

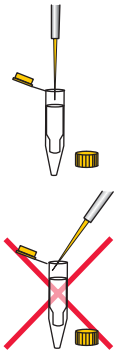
Purification of DNA

12. Transfer a maximum of 100 µL of lysis supernatant containing the DNA into the prepared Spin Column as illustrated:

    Open cap and pipet the sample slowly (5 sec) onto the center of the resin bed of the prepared Spin Column. Close screw cap and loosen again half a turn.

    Important: Do not re-close the screw cap of the Spin Column completely.

    Note: During loading of lysate, do not touch the resin bed with your pipette tip. Residual sample particles may be loaded and will not interfere with purification.



13. Centrifuge for 1 minute at 1,000 x g. The purified DNA flows through the column into the 1.5 mL storage tube. Discard the Spin Column.

The collected DNA can be used immediately or kept at 2-8 °C or for long-term storage at –20 °C. For spectrophotometric analysis, use the 1x Tris Buffer Ⓣ supplied with the kit.

Cap Puncher Protocol

Lysis

1. Perform Standard Protocol steps 1-7.

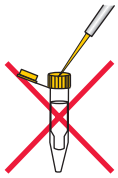
Column Preparation

8. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube. Let stand for 5 to 10 minutes.
9. Use of the Cap Puncher: Punch a hole into the column cap and lift the column together with the Cap Puncher out of the 2 mL collection tube. Snap off bottom closure of the column and detach the Cap Puncher by twisting clockwise while pulling out. Place the punched Spin Column back into the 2 mL reaction tube.
10. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the column buffer.
11. Place the prepared GenElute™-E Spin Column into a new 1.5 mL reaction tube for collection of the purified DNA and place back into the rack.

Purification of DNA

12. Transfer a maximum of 100 µL of lysis supernatant containing the DNA into the prepared Spin Column as illustrated:

- Insert pipet tip vertically through the hole in the column cap.
- Pipet the sample slowly (5 sec) into the column.



Note: Residual cellular debris may be loaded and will not interfere with purification.

13. Centrifuge for 1 minute at 1,000 x g. The purified DNA flows through the column into the 1.5 mL storage tube. Discard the Spin Column.

The collected DNA can be used immediately or kept at 2-8 °C or for long-term storage at –20 °C. For spectrophotometric analysis, use the 1x Tris Buffer Ⓣ supplied with the kit.

Troubleshooting

Problem	Probable Cause	Solution
Low yield	Individual samples have inherent variability Be aware that the DNA yield to be expected is cell type dependent. Optimization needs to be performed by the user to validate for their sample type.	Degraded DNA fragments below < 60 bp are depleted during purification. Using fresh samples stored under appropriate conditions or stabilizing the samples will help to mitigate low sample yields.
	Cell pellet was discarded.	Do not discard the cell pellet and use caution during steps.
	Using too much sample may result in overloading the column's capacity for separation.	Use the recommended sample load. Optimization needs to be performed by the user to validate for their sample type.
	Using too little of sample may result in low sample yields.	Use the recommended sample load. Optimization may need to be performed by the user if their sample type is low yielding.
	Small, possibly degraded, DNA-fragments (< 60 bp) and/or RNA (if RNase was used) are removed during purification. Since these components are co-purified with silica-based kits, there may be artificially lower oD 260 readings with GenElute™-E kits. Thus, the calculation of sample concentration and subsequent yield may appear lower.	Quantitation by measuring signal intensity of bands via gel electrophoresis fragment separation, using a fragment analyzer, or comparing qPCR Ct values will provide a more reliable measurement of full-length gDNA.
	Centrifugation speeds and spin times have been optimized to acquire the fraction of sample containing the nucleic acid.	Verify that centrifugation was performed under the recommended conditions.
	Incomplete lysis of the sample may lead to reduced yields. Lysis times may need to be extended depending on the sample type.	Additionally, the SmartLyse™ enzymes were optimized to work at the recommended temperature. Verify that the heating unit (ex. thermal shaker) is heating correctly.
	If the column preparation steps were performed incorrectly, then the separation resin will be packed incorrectly.	Verify that the preparation steps for the column were performed according to the protocol.
	When performing the Standard Protocol, without the use of the GenElute™-E Single Spin Cap Puncher, the cap may have been left untightened.	Verify that the spin column cap of the column is loosened half a turn to avoid vacuum generation.

Low sample volume	Loading too low of sample or too high of sample may result in sample volume loss. The loaded sample volume is required to be within the recommended range as that volume is required to displace the column buffer.	If the sample volume available to be loaded onto the column is below the recommended range, then bring the sample within the recommended range using lysis buffer. If the sample volume available to be loaded onto the column is above the recommended range, then only load up to the recommended volume.
260/230 ratios appear to be "too low"	In some cases, the 260/230 ratios may be below the recommended range.	Downstream assays have not been shown to be compromised by lower 260/230 ratios using nucleic acid isolated using GenElute™-E kits.
	Using too much volume may result in overloading the column's capacity for separation.	Use the recommended sample load. Optimization needs to be performed by the user to validate for their sample type. Volume loaded can be reduced to a minimum of 50 µl.
RNA residues are observed	If the optional RNase protocol is not performed, then there may be RNA observed.	Perform the optional RNase protocol. Some samples may require an extended incubation due to variability across sample types.
Sample is highly viscous during or after lysis	Using too much sample may result in overloading the column's capacity for separation.	Use the recommended sample load. Optimization needs to be performed by the user to validate for their sample type.
	Genomic DNA are large molecules and sometimes cause a viscous solution.	Shearing sample through extending vortex times throughout the protocol may help reduce sample viscosity.
		Extending the lysis time or adding an 80°C incubation after clearing the sample may help reduce sample viscosity.
Lysate leaks from the hole created by the Cap Puncher during loading	The sample needs to be loaded vertically, allowing the sample to be dispensed correctly into the column. Also, if there is not enough pressure applied using the Cap Puncher then the hole may not be large enough to load the sample.	Apply enough pressure using the Cap Puncher to create a hole and load sample vertically.
Columns with dried resin	In rare cases, the spin columns dry out during storage. This may be due to not storing the columns according to the recommended conditions.	Store GenElute™-E kits according to the recommended conditions.

Product Ordering

Description	Qty	Catalogue No.
GenElute™-E Single Spin Blood DNA Kit	10	EC100-10RXN
	50	EC100-50RXN
	250	EC100-250RXN
GenElute™-E Single Spin Blood DNA High Yield Kit	10	EC200-10RXN
	50	EC200-50RXN
	250	EC200-250RXN
GenElute™-E Single Spin Tissue DNA Kit	10	EC300-10RXN
	50	EC300-50RXN
	250	EC300-250RXN
GenElute™-E Single Spin Cell Culture DNA Kit	10	EC400-10RXN
	50	EC400-50RXN
	250	EC400-250RXN
GenElute™-E Single Spin Plant DNA Kit	10	EC500-10RXN
	50	EC500-50RXN
	250	EC500-250RXN
GenElute™-E Single Spin DNA Cleanup Kit	10	EC600-10RXN
	50	EC600-50RXN
	250	EC600-250RXN
GenElute™-E Organic Solvent DNA Cleanup	10	EC700-10RXN
	50	EC700-50RXN
	250	EC700-250RXN
GenElute™-E Single Spin RNA Cleanup Kit	10	EC800-10RXN
	50	EC800-50RXN
	250	EC800-250RXN
GenElute™-E Tissue Stabilizer	100	EC111-100ML
	500	EC111-500ML
GenElute™-E RNA Gel Loading Buffer	1	EC222-1EA
	5	EC222-5EA
GenElute™-E Single Spin Tissue DNA 96 Kit	2	EC396-2EA
	8	EC396-8EA
GenElute™-E Single Spin Plant DNA 96 Kit	2	EC596-2EA
	8	EC596-8EA
GenElute™-E Single Spin Blood DNA 96 Kit	2	EC196-2EA
	8	EC196-8EA
GenElute™-E Single Spin Cap Puncher	1	EC9999-1EA

Precautions and Disclaimer

This product is for research use only. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Notice

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GenElute™-E Single Spin Checklist for Cell Culture DNA Kit

EC400

Prepare before starting

- ☐ Heat the thermal shaker or heating block/chamber to 60 °C.
- ☐ Set the microcentrifuge to 2,000 x g (cell pelleting) or 1,000 x g (column preparation).

Lysis

- ☐ Transfer up to 1 x 10^6 cultured cells.
- ☐ Centrifuge 1 minute at 2,000 x g to pellet cells. Discard supernatant.
- ☐ Add 55 µL Cell Lysis Buffer LB.
- ☐ Add 25 µL SmartLyse™ C Protease Mix P.
- ☐ Pulse-vortex to resuspend the pelleted cells.

Column prep during lysis

Incubate 10 minutes 60 °C

Thermal Shaker maximum agitation

- ☐ Add 25 µL Clearing Solution C CS and 1 µL RNase A Cell ®.
- ☐ Vortex vigorously with four pulses of 10 seconds each.
- ☐ Incubate for 2 minutes at room temperature.
- ☐ Centrifuge 2 minutes at maximum speed.

Column preparation (during 60 °C incubation)

- ☐ Vortex Spin Column and place in a 2 mL tube.
- ☐ Let stand for 5 to 10 minutes.

Loosen screw cap of Spin Column.

OR

Punch a hole in the cap with the GenElute™-E Single Spin Cap Puncher.

- ☐ Snap off bottom closure. Place Spin Column back into 2 mL tube.
- ☐ Centrifuge 1 minute at 1,000 x g to collect column buffer.
- ☐ Place column in a 1.5 mL tube.

Purification of DNA

- ☐ Transfer lysate supernatant (maximum 100 µL).
- ☐ Centrifuge 1 minute at 1,000 x g to collect DNA.
- ☐ Collected DNA is ready to use.

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