EasySep™ Total Nucleic Acid Extraction Kit

For 75 - 100 preparations using ErythroClear™ Magnet with RNA-specific protocol from STEMprep™ Homogenization samples

Catalog #100-1079

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Description

Isolate total nucleic acid (DNA and RNA) from tissues homogenized using the STEMprep™ Homogenization Protocol (Document #10000033859). Following sample lysis, nucleic acids are captured by silica-coated EasySep™ Total Nucleic Acid Concentrated RapidSpheres™ and separated using ErythroClear™ Magnet (Catalog #01737).

Residual proteins and cell components are removed by washing the separated nucleic acids with 70% ethanol and are released from RapidSpheres™ using an elution buffer. The final isolated fraction contains purified nucleic acids that are immediately available for direct quantification with NanoDrop™ spectrophotometer, additional purification (e.g. DNA removal), or for use in downstream applications.

A DNase I treatment is integrated in RNA Protocol (Table 2) and is suitable for downstream RNA-based applications which require minimal DNA contamination within the extract. Materials required for integrated DNase I treatment are listed in the Materials Required but Not Included.

NOTE: This is the Product Information Sheet (PIS) for extracting total nucleic acids using ErythroClear™ Magnet from tissues homogenized using the STEMprep™ Homogenization Protocol. For extracting nucleic acids from leukapheresis, hPSCs & other 2D-cultured cells, EasySep™-isolated cells, human whole blood and 3D organoids using ErythroClear™ Magnet or 96-Well PCR Microplate Magnet, refer to the applicable PIS (Documents #10000019974/10000019976), available at www.stemcell.com, or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Total Nucleic Acid Concentrated RapidSpheres™	100-1091	1 x 3 mL	Store at 15 - 25°C.	Stable for 12 months from date of manufacture (MFG) on label.	A concentrated suspension of magnetic particles in distilled water.
EasySep™ Total Nucleic Acid Lysis Buffer*	100-1090	1 x 20 mL	Store at 15 - 25°C.	Stable for 12 months from date of manufacture (MFG) on label.	A cell lysis buffer containing a detergent and chaotropic salt.
EasySep™ Total Nucleic Acid Proteinase K*	100-1092	1 x 2 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	A solution containing proteinase K.
EasySep™ Total Nucleic Acid RapidSpheres™ Bottle for Dilution	100-1093	1 Bottle	Not applicable	Not applicable	A sterile, 60 mL bottle for diluting RapidSpheres™

* Refer to the Safety Data Sheet (SDS) for hazard information.

Materials Required but Not Included

PRODUCT NAME	CATALOG #	APPLICABLE PROTOCOL
1.7 mL microcentrifuge tube	e.g. 38089	All protocols
DNase I, RNase-free (1 U/µL)	Thermo Fisher Catalog #EN0521	RNA Protocol (Table 2)
D-PBS (Without Ca++ and Mg++)	37350	All protocols
ErythroClear™ Magnet	01737	All protocols
Ethanol (96 - 100%)*	--	All protocols
Isopropanol (100%)	--	All protocols
Nuclease-Free Water OR Tris-EDTA buffer	79002 OR e.g. IDT 11-05-01-09	All protocols

* Do not use denatured alcohol, which may contain other substances such as methanol or methylethylketone.

Preparation of Reagents and Materials

A. DILUTING EASYSEP™ TOTAL NUCLEIC ACID CONCENTRATED RAPIDSPHERES™

1. Vortex the vial of EasySep™ Total Nucleic Acid Concentrated RapidSpheres™ and transfer 3 mL to EasySep™ Total Nucleic Acid RapidSpheres™ Bottle for Dilution.
2. Add 27 mL of 100% isopropanol to the bottle. The diluted EasySep™ Total Nucleic Acid Concentrated RapidSpheres™ are now ready for use.

   NOTE: If not used immediately, store diluted EasySep™ Total Nucleic Acid Concentrated RapidSpheres™ at room temperature (15 - 25°C). Do not exceed the shelf life of the RapidSpheres™.

B. 70% ETHANOL WASH SOLUTION

Prepare 70% ethanol wash solution as described in the table below. Mix thoroughly.

NOTE: 70% ethanol wash solution must be prepared fresh before performing magnetic nucleic acid extraction. Do not use denatured alcohol, which may contain other substances such as methanol or methylethylketone.

REAGENT	1 SAMPLE	8 SAMPLES + 10% EXCESS
Absolute ethanol	700 µL	6160 µL
Nuclear-Free Water	300 µL	2640 µL

C. DNase I Solution

Genomic DNA is removed from the extract using DNase I, RNase-free (1 U/µL) in RNA Protocol (Table 2). Perform the following preparation steps in an RNase-free work area.

Prepare DNase I solution as described in the table below. Vortex and spin down prior to use. Store on ice.

REAGENT	1 SAMPLE	8 SAMPLES + 10% EXCESS
DNase I, RNase-free (1 U/µL)	10 µL (10 U)	88 µL
10X Reaction Buffer with $MgCl_2$	10 µL	88 µL
Nuclease-Free Water	80 µL	704 µL
Total Volume	100 µL	880 µL

Sample Preparation

See STEMprep™ Homogenization Protocol (Document #10000033859) for sample preparation instructions.

Directions for Use

Refer to Table 1 for detailed instructions for the EasySep™ Total Nucleic Acid Extraction Kit – STEMprep™ Homogenization Standard Protocol. Refer to Table 2 for the EasySep™ Total Nucleic Acid Extraction Kit – STEMprep™ Homogenization RNA Protocol.

Table 1. EasySep™ Total Nucleic Acid Extraction Kit - STEMprep™ Homogenization Standard Protocol

STEP	INSTRUCTIONS	ErythroClear™ (Catalog #01737)
1	Transfer homogenized sample to required tube.	200 µL 1.7 mL microcentrifuge tube (e.g. Catalog #38089)
2	Dilute sample 1:1 with D-PBS (Without Ca++ and Mg++).	200 µL
3	Add Proteinase K to sample.	20 µL
	Mix by pipetting up and down 15 times and incubate in a water bath or heat block.	56°C for 10 minutes
4	Shake the bottle of diluted RapidSpheres™ (Preparation section A). NOTE: Particles should appear evenly dispersed.	20 seconds
	Add diluted RapidSpheres™ to sample.	300 µL
	Mix by pipetting up and down 15 times and incubate.	RT for 5 minutes
5	Place the tube into the magnet and incubate.	RT for 2 minutes
6	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
7	Add 70% ethanol wash solution (Preparation section B). Avoid disturbing the particle pellet.	1 mL
	Incubate.	RT for 1 minute
8	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 3 x 1-minute washes)
10	Allow residual ethanol to evaporate. Do not remove the tube from the magnet. NOTE: Residual ethanol can be aspirated after the first minute.	RT for 2 minutes
11	Remove the tube from the magnet and add elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.	20 - 100 µL**§
	Mix by gently pipetting up and down to fully resuspend the pellet. Incubate. NOTE: Avoid foaming the sample.	RT for 5 minutes
12	Place the tube into the magnet and incubate.	RT for 2 minutes
13	Transfer the supernatant into a new tube.	Extracted nucleic acids are ready for use

RT - room temperature (15 - 25°C)
* Collect the entire supernatant, all at once, into a single pipette.
** To avoid carryover of RapidSpheres™ into the final sample, this volume may be increased to 110 µL. Transfer only 100 µL into the final tube (step 13).
§ Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.

Table 2. EasySep™ Total Nucleic Acid Extraction Kit - STEMprep™ Homogenization RNA Protocol

STEP	INSTRUCTIONS	ErythroClear™ (Catalog #01737)
1	Transfer homogenized sample to required tube	200 µL 1.7 mL microcentrifuge tube (e.g. Catalog #38089)
2	Dilute sample 1:1 with D-PBS (Without Ca++ and Mg++)	200 µL
3	Add Proteinase K to sample.	20 µL
	Mix by pipetting up and down 15 times and incubate.	56°C for 10 minutes
4	Shake the bottle of diluted RapidSpheres™ (Preparation section A). NOTE: Particles should appear evenly dispersed.	30 seconds
	Add diluted RapidSpheres™ to sample.	300 µL
	Mix by pipetting up and down 15 times and incubate.	RT for 5 minutes
5	Place the tube into the magnet and incubate.	RT for 2 minutes
6	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
7	Add 70% ethanol wash solution (Preparation section B). Avoid disturbing the particle pellet.	1 mL
	Incubate.	RT for 1 minute
8	Carefully pipette* off the supernatant. Do not remove the tube from the magnet. Ensure all supernatant is removed.	Discard supernatant
9	Remove the tube from the magnet and resuspend the particle pellet in the DNase I solution (Preparation section C). Note: If particle pellet is difficult to resuspend, use a wider bore tip (i.e. P1000). Avoid foaming the sample.	100 µL
10	Incubate. NOTE: Particles may sink to the bottom of the tube during incubation; this is expected.	RT for 15 minutes
11	Place the tube into the magnet and incubate.	RT for 2 minutes
12	Carefully pipette* (do not pour) the supernatant into a new tube.	100 µL Use a new 1.7 mL tube
13	Add Lysis Buffer to sample.	100 µL
14	Shake the bottle of diluted RapidSpheres™ (Preparation section A). NOTE: Particles should appear evenly dispersed.	20 seconds
15	Add diluted RapidSpheres™ to sample.	150 µL
16	Mix and incubate.	RT for 5 minutes
17	Place the tube into the magnet and incubate.	RT for 2 minutes
18	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
19	Add 70% ethanol wash solution (Preparation section B). Avoid disturbing the particle pellet.	1 mL
	Incubate.	RT for 1 minute
20	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant5,
21	Repeat steps as indicated.	Steps 19 and 20, two more times (total of 3 x 1-minute washes)
22	Allow residual ethanol to evaporate. Do not remove the tube from the magnet. NOTE: Residual ethanol can be aspirated after first minute.	RT for 2 minutes
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STEP	INSTRUCTIONS	ErythroClear™ (Catalog #01737)
23	**Remove the tube from the magnet and add elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.**	20 - 100 µL**§
24	**Mix by gently pipetting up and down to fully resuspend the pellet. Incubate.** **NOTE: Avoid foaming the sample.**	RT for 5 minutes
25	**Place the tube into the magnet and incubate.**	RT for 2 minutes
26	**Transfer the supernatant into a new tube.**	Extracted nucleic acids are ready for use

RT - room temperature (15 - 25°C)
* Collect the entire supernatant, all at once, into a single pipette.
** To avoid carryover of RapidSpheres™ into the final sample, this volume may be increased to 110 µL. Transfer only 100 µL into the final tube (step 26).
§ Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.

Notes and Tips

- Foam generated during the STEMprep™ Homogenization Protocol (Document #10000033859) can be dissipated by placing the STEMprep™ Sample Tube on ice for 20 - 30 minutes until foam has returned to liquid.
- If RapidSpheres™ are still visible in the supernatant after magnetic separation (i.e. the supernatant appears slightly pigmented), each 2-minute magnetic separation step may be extended by an additional 2 minutes (steps 5 and 12 [Table 1] and steps 5, 11, 17 and 25 [Table 2])
- Ensure that the pellet is fully immersed in 70% ethanol wash solution during the wash steps.
- When drying residual ethanol from the pellet, do not exceed 2 minutes as overdrying may result in smaller fragment sizes and increased difficulty when resuspending. An overdried pellet will have a cracked appearance.
- When removing the tube from the magnet prior to addition of the elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer), the pellet will be fixed to the side of the tube. It may be necessary to aspirate and dispense the elution buffer over the pellet several times to ensure that the pellet is fully released from the tube wall.
- When processing a larger number of samples, first add the elution buffer to each tube by pipetting the elution buffer directly onto the particle pellet. After the elution buffer is added to each tube, proceed to resuspending. This avoids pellets overdrying.
- During all elution steps, ensure that the pellet is fully disrupted in the elution buffer. Mix the elution buffer and the pellet by pipetting up and down several times. Thorough mixing of the pellet and elution buffer will ensure efficient release of captured nucleic acids and optimal recovery. Avoid foaming the solution.
- When aspirating the final eluted fraction, there may be residual RapidSpheres™ located at the supernatant meniscus. Avoid aspirating these in the final fraction by positioning the pipette tip at the front of the tube.