EasySep™ Total Nucleic Acid Extraction Kit

For 75 - 100 preparations using ErythroClear™ Magnet with DNA- and RNA-specific protocols

Catalog #100-1079

Document #10000019974 | Version 01

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Description

Isolate total nucleic acid (DNA and RNA) from leukapheresis, whole blood, mouse splenocytes, EasySep™-isolated cells, human pluripotent stem cells (hPSCs), 3D organoids, and other 2D-cultured adherent and non-adherent cells.

EasySep™ Total Nucleic Acid Extraction Kit targets nucleic acids from samples containing up to 1 x 10^6 cells (≤ 5 x 10^6 cells/mL). Following sample lysis, nucleic acids are captured by silica-coated EasySep™ Total Nucleic Acid Concentrated RapidSpheres™ and separated using:

- ErythroClear™ Magnet (Catalog #01737) for standard and whole blood preparations, or
- 96-Well PCR Microplate Magnet (Catalog #100-1304) for preparations in a 96-well format

Residual proteins and cell components are removed by washing the separated nucleic acids with 70% ethanol and are released from the 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.

An optional RNase A treatment can be implemented in both the Standard (Table 1) and Whole Blood Protocols (Table 2), suitable for downstream applications requiring genomic DNA with minimal contamination. A DNase I treatment is integrated in the RNA Protocol (Table 3), which is suitable for downstream RNA-based applications that require minimal DNA contamination within the extract. Materials required for integrated RNase A or 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 with DNA- and RNA-specific protocols. For extracting nucleic acids using 96-Well PCR Microplate Magnet, refer to the applicable PIS (Document #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 3)
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
RNase A, DNase and protease-free (10 mg/mL)	Thermo Fisher Catalog #EN0531	Standard or Whole Blood Protocols with optional RNase treatment (Tables 1 - 2)

*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 the 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 3).

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

NOTE: Prepare DNase I solution in a RNase-free work area.

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

For available fresh and frozen samples, see www.stemcell.com/primarycells.

LEUKAPHERESIS

Pre-processing is not required prior to nucleic acid extraction. Peripheral blood leukapheresis samples can be adjusted to the desired cell concentration with D-PBS (Without Ca++ and Mg++; Catalog #37350) to a maximum concentration of 5 x 10^6 cells/mL.

hPSCs & OTHER 2D-CULTURED CELLS

Adherent cells (e.g. hPSCs): Dissociate cells and quench the dissociation reagent. Proceed with extraction. Cell pelleting and additional washes are not required and may decrease sample purity. If cells are not processed immediately, place on ice until required.

Non-adherent cells: Pellet cells and resuspend in D-PBS (Without Ca++ and Mg++) to the desired starting concentration. Proceed with extraction. If cells are not processed immediately, place on ice until required.

NOTE: For complete instructions on preparing a single-cell suspension of hPSCs, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus (Document #10000007757), available at www.stemcell.com.

NOTE: Cultured cells tested include hPSCs, human multiple myeloma cell line SKMM2, and human breast cancer cell line MCF7.

EASYSEP™-ISOLATED CELLS

Following EasySep™ cell separation, resuspend the isolated cells in D-PBS (Without Ca++ and Mg++) or EasySep™ Buffer (Catalog #20144) at the desired starting concentration. For cells isolated using EasySep™ PBMC Positive or Negative Selection, EasySep™ Direct Cell Isolation, or EasySep™ Release Positive Selection, follow the **Standard Protocol** (Table 1). For cells isolated by EasySep™ Whole Blood Positive Selection, follow the **Whole Blood Protocol** (Table 2).

HUMAN WHOLE BLOOD

Pre-processing is not required prior to nucleic acid extraction. Collect whole blood in a blood collection tube containing heparin, acid-citrate-dextrose solution A (ACDA), or potassium ethylenediaminetetraacetic acid (K-EDTA) anticoagulants. Refer to the **Whole Blood Protocol** (Table 2) for extracting nucleic acids from whole blood.

3D ORGANOIDS

For processing 30 - 50 µL Corning® Matrigel® Matrix (Corning 356231) domes cultured in 24-well plates, first aspirate the medium, then add lysis buffer, proteinase K, and 100 -170 µL PBS directly onto the dome. The volume of PBS added is dependent on the total volume of Corning® Matrigel® Matrix going into the extraction. Add 200 µL PBS minus the total volume of Corning® Matrigel® Matrix across the 1 or 2 domes being processed. Gently triturate the mixture to homogenize the dome (avoid excessive foaming). Once the dome is sufficiently homogenized, transfer the lysate mixture into a microcentrifuge tube and proceed with the 56°C incubation (end of step 3 in Table 1 and 3). Typically 1 - 2 healthy, confluent culture wells can be processed into single extraction. To

process multiple domes into a single extraction, transfer the lysate mixture to the second well and continue gently triturating. For best results, do not attempt to process more than 1 x 10^6 cells per extraction.

Directions for Use

See page 2 for Sample Preparation. Refer to Table 1 and 2 for detailed instructions for the Standard and Whole Blood Protocols, respectively. For the RNA protocol, refer to Table 3.

Table 1. EasySep™ Total Nucleic Acid Extraction Kit - Standard Protocol (100 Preparations)

STEP	INSTRUCTIONS	ErythroClear™ (Catalog #01737)
1	Prepare sample at the indicated cell concentration and volume.	≤ 5 x 10^6 cells/mL 200 µL
2	Add sample to required tube.	1.7 mL microcentrifuge tube (e.g. Catalog #38089)
3	Add Proteinase K to sample.	20 µL
	Add Lysis Buffer to sample.	200 µL
	Mix by pipetting up and down 15 times and incubate in a water bath or heat block.	56°C for 10 minutes
OPTIONAL RNase TREATMENT NOTE: Removal of RNA from the sample may be required for some downstream applications.		---
4	Allow the lysate to cool.	RT for 2 minutes
	Add RNase A, DNase and protease-free (10 mg/mL) to the lysate.	80 µL
	Mix and incubate.	RT for 5 minutes
5	Shake the bottle of diluted RapidSpheres™ (see 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
6	Place the tube into the magnet and incubate.	RT for 2 minutes
7	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
8	Add 70% ethanol wash solution (see Preparation section B). Avoid disturbing the particle pellet.	1 mL
	Incubate.	RT for 1 minute
9	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
10	Repeat steps as indicated.	Steps 8 and 9, two more times (total of 3 x 1-minute washes)
11	Allow residual ethanol in the tube to evaporate. Do not remove the tube from the magnet. NOTE: Residual ethanol can be aspirated after first minute.	RT for 2 minutes
12	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
13	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
14	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 14).
§ 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 - Whole Blood Protocol (75 Preparations)

STEP	INSTRUCTIONS	ErythroClear™ (Catalog #01737)
1	Prepare sample at the indicated start volume.	200 µL
2	Add sample to required tube.	1.7 mL microcentrifuge tube (e.g. Catalog #38089)
3	Add Proteinase K to sample.	20 µL
	Add Lysis Buffer to sample.	200 µL
	Mix and incubate.	56°C for 10 minutes
OPTIONAL RNase TREATMENT NOTE: Removal of RNA from the sample may be required for some downstream applications.		---
4	Allow the lysate to cool.	RT for 2 minutes
	Add RNase A, DNase and protease-free (10 mg/mL) to the lysate.	80 µL
	Mix and incubate.	RT for 5 minutes
5	Shake the bottle of diluted RapidSpheres™ (see 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
6	Place the tube into the magnet and incubate.	RT for 2 minutes
7	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
8	Remove the tube from the magnet and add elution buffer** (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.	100 µL
	Mix by gently pipetting up and down 2 - 3 times and incubate.	RT for 5 minutes
9	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
10	Carefully pipette* (do not pour) the supernatant into a new tube.	100 µL Use a new 1.7 mL tube
11	Add Lysis Buffer to sample.	100 µL
12	Shake the bottle of diluted RapidSpheres™ (see Preparation section A). NOTE: Particles should appear evenly dispersed.	20 seconds
	Add diluted RapidSpheres™ to sample.	150 µL
	Mix and incubate.	RT for 5 minutes
13	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
14	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
15	Add 70% ethanol wash solution (see Preparation section B). Avoid disturbing the particle pellet.	1 mL
	Incubate.	RT for 1 minute
16	Carefully pipette* off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
17	Repeat steps as indicated.	Steps 15 and 16, two more times (total of 3 x 1-minute washes)
18	Allow residual ethanol in the tube to evaporate. Do not remove the tube from the magnet. NOTE: Residual ethanol can be aspirated after first minute.	RT for 2 minutes
Continue on to next page.		Continue on to next page.

STEP	INSTRUCTIONS (CONTINUED)	ErythroClear™ (Catalog #01737) 
19	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
20	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
21	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 21).
§ Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.

Table 3. EasySep™ Total Nucleic Acid Extraction Kit - RNA Protocol (75 Preparations)

STEP	INSTRUCTIONS	ErythroClear™ (Catalog #01737)
1	Prepare sample at the indicated cell concentration and volume.	≤ 5 x 10^6 cells/mL 200 µL
2	Add sample to required tube.	1.7 mL microcentrifuge tube (e.g. Catalog #38089)
3	Add Proteinase K to sample.	20 µL
	Add Lysis Buffer to sample.	200 µL
	Mix by pipetting up and down 15 times and incubate.	56°C for 10 minutes
4	Shake the bottle of diluted RapidSpheres™ (see 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 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 (see Preparation section B).	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 (see Preparation section C). Note: If particle pellet is difficult to resuspend, use a wider bore tip (e.g. 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 (with lid open) 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™ (see Preparation section A). NOTE: Particles should appear evenly dispersed.	20 seconds
Continue on to next page.		Continue on to next page.

STEP	INSTRUCTIONS (CONTINUED)	ErythroClear™ (Catalog #01737) 
15	Add diluted RapidSpheres™ to sample.	150 µL
16	Mix and incubate.	RT for 5 minutes
17	Place the tube (with lid open) 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 (see 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 supernatant
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
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 (with lid open) 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

- If needed, samples can be frozen at -20°C for downstream DNA applications and at -80°C for downstream RNA applications following the 56°C incubation (Table 1 - 3, step 3). To complete the extraction, thaw the frozen samples on ice and proceed with the rest of the protocol.
- 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.
- 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 more than 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, 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.

Data

Nucleic acid extractions for hPSCs, leukapheresis, mouse splenocytes, peripheral blood mononuclear cells (PBMCs; isolated using EasySep™ Human CD3 Positive Selection Kit II [Catalog #17851]) were performed following the Standard Protocol (Table 1).

Whole blood extractions were performed following the Whole Blood protocol (Table 2). The data shown represent mean ± SD.

Table 4. Nucleic Acid Recovery and Purity

STARTING SAMPLE	NORMALIZED RECOVERY (µg/1,000,000 cells)	RECOVERY (ng/µL)	PURITY (260/280)	PURITY (260/230)
hPSCs (n = 4)	18 ± 1.3	N/A	2 ± 0.01	2 ± 0.02
Leukapheresis (n = 3)	5.9 ± 0.28	N/A	1.9 ± 0.00	1.9 ± 0.07
Mouse splenocytes (n = 3)	3.7 ± 0.50	37 ± 5.0	1.9 ± 0.01	1.9 ± 0.16
EasySep™-isolated PBMCs (n = 4)	4.8 ± 1.0	48 ± 10	1.9 ± 0.01	2.1 ± 0.17
Whole blood (n = 7)	4.3 ± 0.8	43 ± 7.67	1.9 ± 0.04	2.1 ± 0.20

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