EasySep™ Total Nucleic Acid Extraction Kit

For 75 - 100 preparations using the ErythroClear™ Magnet

Catalog #100-1079

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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), and other 2D-cultured adherent and non-adherent cells.

The EasySep™ Total Nucleic Acid Extraction Kit targets nucleic acids from cell suspensions containing up to 1 x 10^6 cells (≤ 5 x 10^6 cells/mL). Following sample lysis, nucleic acids are captured by EasySep™ Total Nucleic Acid RapidSpheres™ and separated using the ErythroClear™ Magnet (Catalog #01737) for standard and whole blood preparations, or the 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 the NanoDrop™ spectrophotometer, additional purification (e.g. DNA removal), or for use in downstream applications.

NOTE: This is the Product Information Sheet (PIS) for extracting total nucleic acids using the ErythroClear™ Magnet. For extracting nucleic acids using the 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 #
ErythroClear™ Magnet	01737
1.7 mL microcentrifuge tube	e.g. 38089
Ethanol (96 - 100%)*	--
Isopropanol (100%)	--
Nuclease-Free Water OR Tris-EDTA buffer	79002 OR e.g. IDT 11-05-01-09

*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 RAPIDSPHERES™

1. Vortex the vial of Concentrated EasySep™ Total Nucleic Acid 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 RapidSpheres™ are now ready for use.

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

B. 70% ETHANOL WASH SOLUTION

To prepare 3.3 mL of 70% ethanol wash solution, add 990 µL of water to 2.31 mL of absolute ethanol. 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.

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, 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 by 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.

Directions for Use

See page 2 for Sample Preparation. Refer to Tables 1 and 2 for detailed instructions for the Standard and Whole Blood Protocols, respectively.

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 RNaseA, DNase and protease-free (10 mg/mL; Thermo Fisher Catalog #EN0531) to the lysate.	40 µL
	Mix and incubate.	RT for 2 minutes
5	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 and incubate.	RT for 5 minutes
6	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
7	Carefully pipette* (do not pour) off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
8	Add 70% ethanol wash solution (Preparation section B).	1 mL
	Incubate.	RT for 1 minute
9	Carefully pipette* (do not pour) 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 separations)
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.	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	Carefully pipette* (do not pour) 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.
** If DNase treatment is required post-extraction, elute in Nuclease-Free Water.
*** 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).

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
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 and incubate.	RT for 5 minutes
5	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
6	Carefully pipette* (do not pour) off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
7	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
8	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
9	Carefully pipette* (do not pour) the supernatant into a new tube.	100 µL Use a new 1.7 mL tube
10	Add Lysis Buffer to sample.	100 µL
11	Shake the bottle of diluted RapidSpheres™ (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
12	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
13	Carefully pipette* (do not pour) off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
14	Add 70% ethanol wash solution (Preparation section B).	1 mL
	Incubate.	RT for 1 minute
15	Carefully pipette* (do not pour) off the supernatant. Do not remove the tube from the magnet.	Discard supernatant
16	Repeat steps as indicated.	Steps 14 and 15, two more times (total of 3 x 1-minute separations)
17	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
18	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 to fully resuspend the pellet. Incubate. NOTE: Avoid foaming the sample.	RT for 5 minutes
19	Place the tube (with lid open) into the magnet and incubate.	RT for 2 minutes
20	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.
** If DNase treatment is required post-extraction, elute in Nuclease-Free Water.
*** 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).

Optional DNase Treatment

Genomic DNA may be removed from the extracted nucleic acids using DNase I, RNase-free (1 U/µL; e.g. Thermo Fisher Catalog #EN0521). Perform the following steps in an RNase-free work area. The following protocol is for treating 1 µg of extracted nucleic acids. If scaling up the reaction, adjust volumes accordingly.

1. Add 1 U of DNase I, RNase-free (1 U/µL) to 1 µg of extracted nucleic acids.
2. Add 1 µL of 10X Reaction Buffer with $MgCl_2$ (i.e. 100 mM Tris-HCl [pH 7.5 at 25°C], 25 mM $MgCl_2$, and 1 mM $CaCl_2$) and top up with DEPC-treated water (e.g. Thermo Fisher Catalog #R0601) to a total volume of 10 µL.
3. Incubate at 37°C for 30 minutes.
4. Add 1 µL of 50 mM EDTA and incubate at 65°C for 10 minutes in a heat block.

Purified nucleic acid sample is ready for use in downstream applications.

Notes and Tips

- Ensure that the pellet is fully immersed in 70% ethanol wash solution during the wash steps.
- 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 elution buffer over the pellet several times to ensure that all material is released from the tube wall.
- 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 of 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.
- When drying residual ethanol from the pellet (i.e. Table 1 step 11, Table 2 step 17), do not exceed more than two minutes as over-drying may result in smaller fragment sizes (pellet will take on a 'cracked' appearance).
- If a higher concentration is desired, the elution volume may be decreased to a minimum of 20 µL.
- If RapidSpheres™ still are 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.

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 3. 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