EasySep™ Total Nucleic Acid Extraction Kit

For 300 - 400 preparations using 96-Well PCR Microplate Magnet with DNA- and RNA-specific protocols

Catalog #100-1079

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Description

Isolate total nucleic acid (DNA and RNA) from leukapheresis samples, EasySep™-isolated cells, human pluripotent stem cells (hPSCs), and other 2D cultured cells. 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 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 a NanoDrop™ spectrophotometer, additional purification (e.g. DNA removal), or for use in downstream applications.

An optional RNase A treatment can be implemented in the Standard protocol (Table 1) and is suitable for downstream applications requiring genomic DNA with minimal contamination. A DNase I treatment is integrated in the RNA protocol (Table 2), 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 treatments are listed in the Materials Required but Not Included.

NOTE: This is the Product Information Sheet (PIS) for extracting total nucleic acids using the 96-Well PCR Microplate Magnet with DNA- and RNA-specific protocols. For extracting nucleic acids using the ErythroClear™ Magnet, refer to the applicable PIS (Document #10000019974), 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 2 years 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 2 years 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
96-Well PCR Microplate	100-1304	All protocols
Axygen® PCR Microplate (96-Wells, Half Skirt, Single Notch)	38103	All protocols
DNase I, RNase-free (1 U/µL)	Thermo Fisher Catalog #EN0521	RNA Protocol (Table 2)
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 Protocol with optional RNase treatment (Table 1)

* 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 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	140 µL	1232 µL
Nuclease-Free Water	60 µL	528 µL

C. DNase I SOLUTION

Genomic DNA is removed from the extract using DNase I, RNase-free (1 U/µL) in the RNA Protocol (Table 2).

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

NOTE: Perform the following preparation steps in an RNase-free work area.

REAGENT	1 SAMPLE	8 SAMPLES + 10% EXCESS
DNase I, RNase-free (1 U/µL)	2.5 µL (2.5 U)	22 µL
10X Reaction Buffer with $MgCl_2$	2.5 µL	22 µL
Nuclease-Free Water	20 µL	176 µL
Total Volume	25 µL	220 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 AND 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) to 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).

Directions for Use

See page 2 for Sample Preparation. Refer to Table 1 for detailed instructions for the Standard Protocol. A multi-channel pipettor is recommended when performing this protocol. For the RNA protocol, refer to Table 2.

Table 1. EasySep™ Total Nucleic Acid Extraction Kit - Standard Protocol (400 Preparations)

STEP	INSTRUCTIONS	96-Well PCR Microplate (Catalog #100-1304) 
1	Prepare sample at the indicated cell concentration and volume.	≤ 5 x 10^6 cells/mL 50 µL
2	Add sample to each well of the plate.	Axygen® PCR Microplate (96-Wells, Half Skirt, Single Notch) (Catalog #38103)
3	Add Proteinase K to each well.	5 µL
	Add Lysis Buffer to each well.	50 µL
	Mix by pipetting up and down 15 times and incubate in a thermal cycler 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.	20 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 each well.	75 µL
	Mix by pipetting up and down 15 times and incubate.	RT for 5 minutes
6	Place the plate into the magnet and incubate.	RT for 2 minutes
7	Carefully pipette* off the supernatant. Do not remove the plate from the magnet.	Discard supernatant
8	Add 70% ethanol wash solution (see Preparation section B). NOTE: Dispense ethanol down the sides of the wells, avoiding the pellets.	200 µL
	Incubate.	RT for 1 minute
9	Carefully pipette* off the supernatant. Do not remove the plate 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 wells to evaporate. Do not remove the plate from the magnet. NOTE: Residual ethanol can be aspirated after the first minute.	RT for 2 minutes
12	Remove the plate from the magnet and add elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellets.	5 - 25 µL**§
	Mix by gently pipetting up and down to fully resuspend the pellets. Incubate. NOTE: Avoid foaming the sample.	RT for 5 minutes
13	Place the plate into the magnet and incubate.	RT for 2 minutes
14	Transfer the supernatant into a new plate.	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 27 µL. Transfer only 25 µL into the final plate (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 - RNA Protocol (300 Preparations)

STEP	INSTRUCTIONS	96-Well PCR Microplate (Catalog #100-1304) 
1	Prepare sample at the indicated volume.	≤ 5 x 10^6 cells/mL 50 µL
2	Add sample to each well of the plate.	Axygen® PCR Microplate (96-Wells, Half Skirt, Single Notch) (Catalog #38103)
3	Add Proteinase K to each well.	5 µL
	Add Lysis Buffer to each well.	50 µL
	Mix by pipetting up and down 15 times and incubate in a thermal cycler or heat block.	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 each well.	75 µL
	Mix by pipetting up and down 15 times and incubate.	RT for 5 minutes
5	Place the plate into the magnet and incubate.	RT for 2 minutes
6	Carefully pipette* off the supernatant. Do not remove the plate from the magnet.	Discard supernatant
7	Add 70% ethanol wash solution (see Preparation section B). NOTE: Dispense ethanol down the sides of the wells, avoiding the pellets.	200 µL
	Incubate.	RT for 1 minute
8	Carefully pipette* off the supernatant. Do not remove the plate from the magnet. Ensure all supernatant is removed.	Discard supernatant
9	Remove the plate 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 (i.e. P1000). Avoid foaming the mixture.	25 µL
10	Incubate. NOTE: Particles may sink to the bottom of the plate during incubation; this is expected.	RT for 15 minutes
11	Place the plate into the magnet and incubate.	RT for 2 minutes
12	Carefully pipette the supernatant into a new plate.	25 µL Use a new PCR microplate
13	Add Lysis Buffer to each well.	25 µL
14	Shake the bottle of diluted RapidSpheres™ (see Preparation section A). NOTE: Particles should appear evenly dispersed.	20 seconds
15	Add diluted RapidSpheres™ to each well.	37.5 µL
16	Mix and incubate.	RT for 5 minutes
17	Place the plate into the magnet and incubate.	RT for 2 minutes
18	Carefully pipette* off the supernatant. Do not remove the plate from the magnet.	Discard supernatant
19	Add 70% ethanol wash solution (see Preparation section B). Avoid disturbing the particle pellet.	200 µL
20	Incubate.	RT for 1 minute
	Carefully pipette* off the supernatant. Do not remove the plate from the magnet.	Discard supernatant
21	Repeat steps as indicated.	Steps 19 and 20, two more times (total of 3 x 1-minute washes)
Continue on to next page.		Continue on to next page.

STEP	INSTRUCTIONS (CONTINUED)	96-Well PCR Microplate (Catalog #100-1304) 
22	**Allow residual ethanol to evaporate. Do not remove the plate from the magnet.** **NOTE: Residual ethanol can be aspirated after the first minute.**	RT for 2 minutes
23	**Remove the plate from the magnet and add elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.**	5 - 25 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 plate (with lid open) into the magnet and incubate.**	RT for 2 minutes
26	**Transfer the supernatant into a new plate.**	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 27 µL. Transfer only 25 µL into the final plate (step 26).
§ Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.

Notes and Tips

- For improved ease-of-use and better control when dispensing and resuspending, use an 8-channel pipettor (e.g. Catalog #38110) instead of a 12-channel pipettor.
- When performing total nucleic acid extraction in a 96-well PCR microplate format, use non-skirted or half-skirted microplates, as full-skirted plates may obscure the user's view of the sample pellet.
- 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 (Tables 1 - 2, 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.
- When pipetting small volumes into the plate (i.e. proteinase K), pipette against the same side of each well; this allows the user to visually confirm which wells have been filled.
- Ensure that the pellets are fully immersed in 70% ethanol wash solution during the wash steps.
- During the ethanol washing steps, use a slight forward-and-back rocking motion of the multi-channel pipettor when aspirating the residual ethanol. This ensures that each pipette tip makes contact with the bottom of each well in order to successfully remove ethanol prior to elution.
- 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 over-dried pellet will have a cracked appearance.
- When the plate is removed from the magnet prior to addition of the elution buffer, the pellet will be fixed to the side of the wells. The elution buffer may be dispensed over the pellet to release it. It may be necessary to aspirate and dispense the elution buffer over the pellet several times to ensure all material is released from the well.
- When processing a larger number of samples, first add the elution buffer to each sample by pipetting the elution buffer directly onto the particle pellet. After the elution buffer is added to each sample, proceed to resuspending. This avoids pellets overdrying.
- 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 opposite the pellet.

Data

Data shown represent mean ± SD.

Table 3. Nucleic Acid Recovery and Purity

STARTING SAMPLE	NORMALIZED RECOVERY (µg/1,000,000 cells)	PURITY (260/280)	PURITY (260/230)
hPSCs (n = 32)	12 ± 1.8	2.1 ± 0.02	2.1 ± 0.28
Leukopak (n = 12)	6 ± 0.54	2 ± 0.05	2.2 ± 0.14