EasySep™ Total Nucleic Acid Extraction Kit

For 400 preparations using the 96-Well PCR Microplate Magnet

Catalog #100-1079

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Description

Isolate total nucleic acid (DNA and RNA) from human pluripotent stem cells (hPSCs) and leukapheresis samples.

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 a 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 96-Well PCR Microplate Magnet. 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 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 #
96-Well PCR Microplate	100-1304
Axygen® PCR Microplate (96-Wells, Half Skirt, Single Notch)	38103
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 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 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.

HUMAN PLURIPOTENT STEM CELLS (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.

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.

EASYSEP™-ISOLATED CELLS

Following EasySep™ cell separation, resuspend the isolated cells in D-PBS (Without Ca++ and Mg++; Catalog #37350) or EasySep™ Buffer (Catalog #20144) to the desired starting concentration.

Directions for Use

See page 2 for Sample Preparation. A multi-channel pipettor is recommended when performing this protocol.

Table 1. EasySep™ Total Nucleic Acid Extraction Kit

STEP	INSTRUCTIONS	96-Well PCR Microplate (Catalog #100-1304) 
1	Prepare sample at the indicated cell concentration within the volume range.	≤ 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.	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; Thermo Fisher Catalog #EN0531) to the lysate.	4 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 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 (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* (do not pour) 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 separations)
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.	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 96-well plate into the magnet and incubate.	RT for 2 minutes
14	Carefully pipette* the supernatant into a new 96-well 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.
** 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 30 µL. Transfer only 25 µL to each well of the new plate (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 pellets are fully immersed in 70% ethanol wash solution during the wash steps.
- When performing total nucleic acid extraction in a 96-well PCR plate format, use non-skirted or half-skirted microplates, as full-skirted plates may obscure the user's view of the sample pellet.
- For improved ease-of-use and better control when dispensing and resuspending, use an 8-channel multi-pipette instead of a 12-channel multi-pipette.
- 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.
- 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 on the magnet before elution, do not exceed two minutes of incubation as over-drying may result in smaller fragment sizes (i.e. pellet will take on 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 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.
- 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.

Data

Data shown represent mean ± SD.

Table 2. 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