Datasheet

EXO-DNA

Circulating and EV-associated DNA extraction kit

This product is for research use only.
It is highly recommended to read this users guide in its entirety prior to using this product.
Do not use this kit or its components beyond the indicated expiration date.

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PRODUCT DESCRIPTION

Product overview

Together with RNAs, genomic single or double-stranded DNA and mitocondrial DNA have been recently detected in exosomes and microvesicles. In particular the majority of double-stranded DNA seems to be associated with tumor derived exosomes and can become and important new source of biomarkers for tumor detection (Thakur, et al. «Double-stranded DNA in exosomes: a novel biomarker in cancer detection.» 2014. Kahlert et al. «Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer.» 2014) .

EXO-DNA combine the ability to isolate EVs and ciculating DNA from a wide range of biofluids (plasma, urine, serum etc.) or culture supernatants with a user friendly system of DNA purification. Isolated EVs are lysed with the appropriate lysis buffer and DNA is purified by spin columns and optimized buffers with a fast turnaround time. In addition EXO-DNA Kit provides lyophilized exosomes to be used as quality controls for exosome capture and DNA extraction. Procedure takes about 1 hour and 45 minutes for yielding purified genomic DNA ready for downstream analysis such as PCR, RT-PCR, qRT-PCR and microarrays.

About Exosomes

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

DNA extraction kit available:

- EXO-DNA Plasma and Serum: Isolation of circulating and EV-associated DNA from plasma and serum samples

- EXO-DNA Urine and Cell media: Isolation of circulating and EV-associated DNA from urine and cell media samples

PRODUCT CONTENT

Kit	Component	Description	Amount
EXO-DNA Plasma and Serum	EXO-Prep	Reagent for EVs isolation from biofluids	1 bottle (3 ml, 20 reactions) 1 bottle (5 ml, 40 reactions)
Exo-DNA Urine and Cell Media	EXO-Prep	Reagent for EVs isolation from biofluids	1 bottle (21 ml, 20 reactions) 1 bottle (42 ml, 40 reactions)
EXO-DNA Plasma and Serum	Exosome Standards for positive control	Human plasma or serum Exosome Standards	1 vial (100 µg)
Exo-DNA Urine and Cell Media	Exosome Standards for positive control	Human urine or cell derived Exosome Standards	1 vial (100 µg)
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Lysis buffer	Solution for exosome lysis	1 bottle (5 ml, 20 reactions) 1 bottle (9 ml, 40 reactions)
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Proteinase K	Reagent for protein digestion	1 vial (450 µl) 20 reactions 2 vial (450 µl) 40 reactions
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Washing Buffer 1	Solution for column washing	1 bottle (6 ml; to add 10 ml of Ethanol 96%) 20 reactions 2 bottles (6 ml; to add 10 ml of Ethanol 96%) 40 reactions
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Washing Buffer 2	Solution for column washing	1 bottle (5 ml; to add 12 ml of Ethanol 96%) 20 reactions 2 bottles (5 ml; to add 12 ml of Ethanol 96%) 40 reactions
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Elution buffer	Buffer for column elution	1 vial (1,5 ml)
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Columns	Columns for RNA extraction (assembled with one tube)	22 columns (20 reactions) 42 columns (40 reactions)
EXO-DNA Plasma and Serum Exo-DNA Urine and Cell Media	Elution tubes	RNase free microfuge tubes (1.5mL) for Elution	22 tubes (20 reactions) 42 tubes (40 reactions)

Other material required

- Single-use and/or pipettes with disposable tips 2-100 µl
- Pipettes 1 ml and 5 ml for reagent preparation
- PBS
- Disposable pipetting reservoirs
- Ethanol 96%

STORAGE INFORMATION

All reagents provided within the Exosome Total RNA Extraction Kit can be stored at +4°C for up to 1 year.

Open and reconstituted components	
DNA-Prep	Store at +4°C for up 1 year
Exosome standards	The remaining reconstituted standard stock solution should be aliquoted into polypropylene vials (preferably low binding) and stored at -20°C for up to one month or at -80°C for up to six months. Strictly avoid repeated freeze-and-thaw cycles
Columns, Elution tubes	Columns and tubes can be store at room temperature as well as at +4°C
Lysis buffer	Store at +4°C
Washing Buffer 1	
Washing Buffer 2	
Proteinase K	
Elution buffer	

PROCEDURE FOR DNA EXTRACTION

Starting volume

Fluid	Volume suggested
Plasma	500 µl
Serum	500 µl
Cell Medium	1 ml
Urine	5 ml

STEP A: Sample preparation:

Prepare the sample by centrifugation steps as suggested in the table below:

Fluid	Suggested	Optional
Plasma	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate vesicles > 200 nm)
Serum	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate vesicles > 200 nm)
Urine	10 min at 300 g (save super) Concentrate 10 fold in MWCO concentrator.	
Cell media	10 min at 300 g (save super) 20 min at 1200 g (save super) Concentrate 10 fold in MWCO concentrator	Centrifuge before MWCO concentration, 30 min at 10000 g (to eliminate vesicles > 200 nm).

STEP B: Reagent preparation

- **Washing Buffer 1** and **Washing buffer 2**
  - Add the volume of pure ethanol (96%) indicated on the label of the bottlles of both Washing Buffers.
- **DNA-Prep, Elution buffer and Lysis buffer are ready to use.**
- **Lyophilized Exosome Standards**
  - Reconstitute lyophilized exosome standard by adding 100 µl of deionized water and pipetting the solution up and down 10-15 times, avoiding bubbles. Vortex the reconstituted standard for 60 seconds. Briefly centrifuge the tubes containing the standard to ensure that the solution is collected at the bottom of the tube.

STEP C: Circulating DNA and EV isolation

From Plasma and Serum

- Add 125 µl of DNA-Prep reagent (ratio DNA-Prep/Sample 1/4) to 500 µl of precleared sample
- Mix well by pipetting and inverting the tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at +4°C as well as at RT)
- Discard the supernatant
- Eliminate the remaining supernatant into the tube with a tip
- Resuspend isolated exosomes in 200 µl of PBS 1X

From Urine

- Add 1 ml of DNA-Prep reagent to 4 ml of precleared urine
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant
- Centrifuge for 2 minutes at 1500 g to eliminate entirely the supernatant
- Resuspend isolated exosomes in 100 µl of PBS 1X

From Cell Media

- Add 1 ml of DNA-Prep reagent to 1 ml of precleared cell medium
- Mix well by pipetting and inverting tube
- Incubate on ice for 1 hour
- Centrifuge 20 minutes at 10000 g (centrifuge can be performed at 4°C or at RT)
- Discard the supernatant

- Centrifuge for 2 minutes at 1500 g to eliminate entirely the supernatant
- Resuspend the pellet in 100 µl of PBS 1X

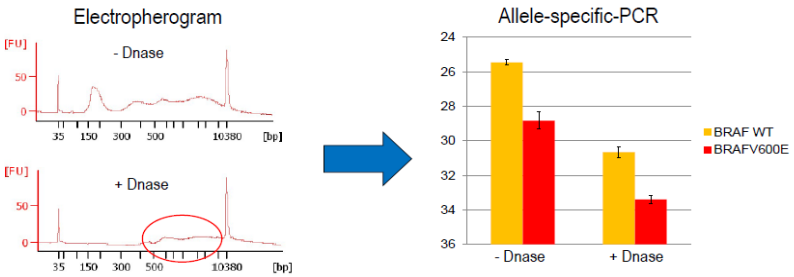
STEP D: DNA Extraction

- **LYSIS**
  - Add 20 µl of Proteinase K (600 mAU/ml)
  - Add 200 µl of Lysis Buffer
  - Mix well by vortexing 30 seconds.
  - Incubate samples at 56°C for 10 minutes.

- **DNA PURIFICATION**
  - Add 200 ul of Ethanol 96% and mix by inverting the tube 6-8 times
  - Transfer the mixture in a Spin Column and centrifuge at 10000 g for 1 minute. Discard the flow-through
  - Add 500 µl of Washing Buffer 1, centrifuge for 1 minute and discard the flow-through
  - Add 500 µl of Washing Buffer 2, centrifuge for 1 minute and discard the flow-through
  - Centrifuge 2 additional minutes at 16000 g.
  - Transfer the spin column to an Elution Tube
  - Elute the DNA from the column adding 50 µl of Elution Buffer
  - Incubate for 5 minutes at room temperature
  - Centrifuge 1 minute at 200 g
  - Centrifuge 1 minute at 16000 g

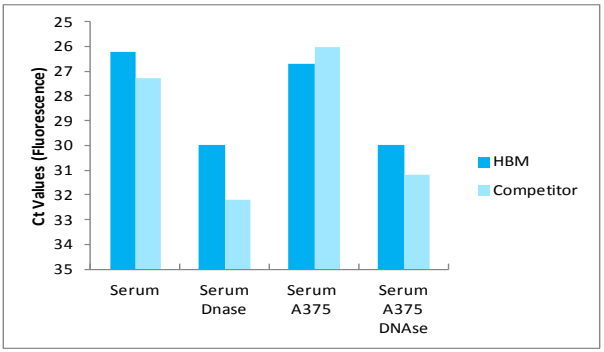
DNA ANALYSIS

EV associated DNA is suitable for point mutation analysis by allele-specific PCR



Healthy donor serum was spiked with 100 µg of purified exosome from BRAFV600E-positive A375 melanoma cell lines. Vesicles were isolated by chemical precipitation with DNA-Prep and treated with or without Dnase 1, to distinguish circulating + EV related and only EV related DNA. Following digestion, DNA was extracted with Exo-DNA kit and analyzed by allele-specific PCR.

EXO-DNA Kit guarantees high efficiency isolation of circulating and EV-associated DNA



Amplification of beta-actin from exosome-derived DNA. Exosomes were isolated from serum with our without artificial spike (A375-derived exosomes) using DNA-Prep solution and treated (or not) with DNAse I. DNA was extracted with HBM EXO-DNA kit and competitor and beta actin was amplified by qPCR.

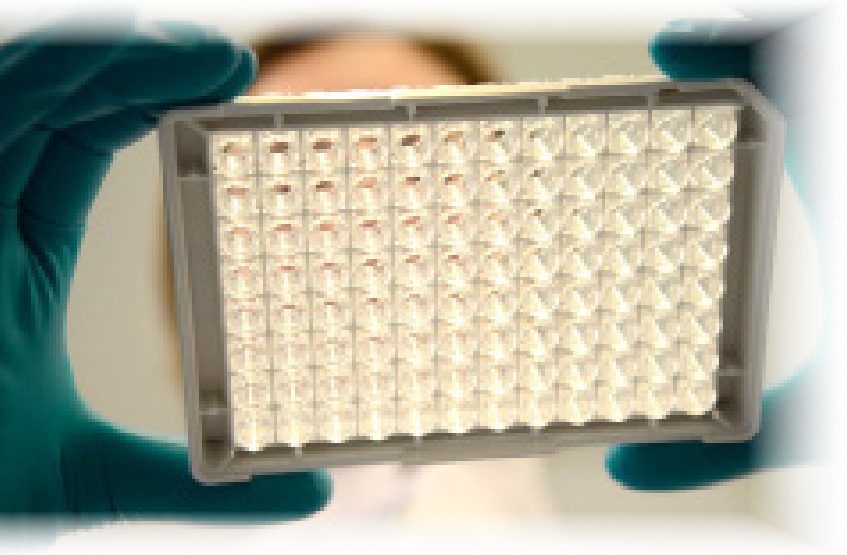
TROUBLESHOOTING	
Problem/ Possible Cause	**Suggested Solution**
Low DNA yield	- Be sure to add Proteinase K in the mixture of lysis buffer - Increase the incubation at RT during the elution step - Do not use water to elute DNA but use only the Elution buffer provided in the kit
DNA is sheared or degradeted	- Avoid to mix the lysate too vigorously - Avoid to form bubbles during mixing steps - Do not touch the membrane of the column with the tip - Treatment with Dnase must be done before to lyse the vesicles. Be careful to disactivate the Dnase before to proceed to the lysis. - Avoid repeated freeze and thaw cycles
Incomplete elution	Prolong the incubation time with Elution Buffer to 5-10 min or repeat elution step once again (25 µl + 25 µl)
Ethanol contamination	After the second washing step, centrifuge once again for 2 mintes at 15000 g Dry the membrane of the column by incubation at RT (no flow hood)

Info and technical support: info@hansabiomed.eu

RELATED PRODUCTS

As the first company entirely dedicated to the field of exosomes, we offer a panel of Kits and Reagents for Exosome Research.
Ordering information on a variety of reagents and apparatus available from HansaBioMed is provided below. For more information, visit our website at www.hansabiomed.eu.

Products	Quantity	Catalog Number
ExoTEST™ Ready To Use Kit for Overall Exosome capture and quantification from Biological fluids	Ready to Use Kit	HBM-RTK-POF/##
ExoTEST™ Ready To Use Kit for Overall Exosome capture and quantification from Cell culture supernatant	Ready to Use Kit	HBM-RTK-POC/##
ExoTEST™ Ready To Use Kit for Tumor-derived Exosome enrichment and quantification from Biological fluids	Ready to Use Kit	HBM-RTK-PTF/##
Exosome Total RNA Extraction Kit (Immunobeads, 10 or 20 Reactions)	Ready to Use Kit	HBM-RNA-BOF-##/#
Tumor-derived Exosome Total RNA Extraction Kit (Immunobeads, 10 or 20 Reactions)	Ready to Use Kit	HBM-RNA-BTF-##/#
Immunoplates for Overall Exosome capture from Biological fluids	96 wells plate	HBM-POF-##/##
Immunoplates for Overall Exosome capture from Cell culture supernatant	96 wells plate	HBM-POC-##/##
Immunoplates for Tumor-derived Exosome capture and enrichment from Biological fluids	96 wells plate	HBM-PTF-##/##
Immunoplates for Neural-derived Exosome capture and enrichment from Biological fluids	96 wells plate	HBM-PNF-##/##
Immunoplates for Glial-derived Exosome capture and enrichment from Biological fluids	96 wells plate	HBM-PGF-##/##
Immunoplates for Monocytes- and Platelets-derived Exosome capture and enrichment from Plasma samples	96 wells plate	HBM-PPP-##/##
Immunobeads for Overall Exosome capture from Biological fluids - 0.4, 1 or 4 microns immunobeads size - Simple or Covalent coating	10 or 20 reactions	HBM-BOLF-##/##
Immunobeads for Overall Exosome capture from Cell culture supernatant - 0.4, 1 or 4 microns immunobeads size - Simple or Covalent coating	10 or 20 reactions	HBM-BOLC-##/##
Immunobeads for Tumor-derived Exosome capture and enrichment from Biological fluids - 0.4, 1 or 4 microns immunobeads size - Simple or Covalent coating	10 or 20 reactions	HBM-BTLF-##/##



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