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Cleanascite™

Lipid adsorption and clarification reagent

- Effectively replaces chlorinated/fluorinated hydrocarbons (eg. freon)
- Workflows for antibodies, proteins, nucleic acids, proteoglycans, and most serum analytes
- A high binding capacity for lipids with minimal cross-reactivity with proteins and nucleic acids
- Ideal for clarifying ascites, serum, cell & tissue culture, bile, saliva, fecal and organ homogenates
- Simple microfuge (not ultra) centrifugation protocols
- Exquisite selectivity profile including extracellular vesicle and exosome clearance
- Compatible with cell response assays
- For bioprocessing, extends the life of membrane and chromatographic columns

Cleanascite™ selectively removes lipids, cell debris, lipoproteins, floating fats, impurities from Cohn paste, transgenic milk, egg yolk and biological samples for pretreatment of samples prior to purification. The reagent is a solid-phase, non-ionic adsorbent supplied as a suspension in saline, ready for use. Simply add, centrifuge and/or filter. The clarified supernatant is ready for subsequent downstream processing or analysis.

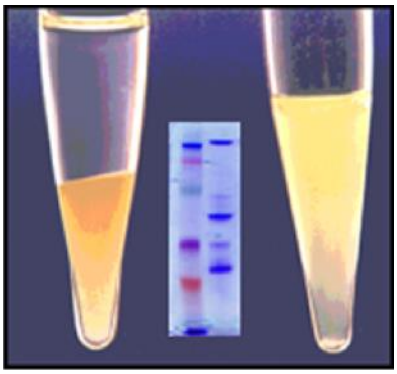
Removes Lipid Factors
Phospho-Lipids
>99% Cholesterol &
Triglycerides
Lipoproteins
Extracellular Vesicles
(Exosomes)

Cleanascite™
Solid-phase Aqueous Separation
No Solvents, Freon or Chloroform
Simple Centrifuge (Not Ultra) Protocols
Safe Disposal

Improved Assay Performance

- **ELISA**
- **Immunocapture Microarrays**
- **LC-MS**
- **Toxin Neutralizing Titer**
- **Cell Response**

Egg Yolk After (Left) and Before (Right) Treatment With Cleanascite™



Insert: PAGE showing
Left: Markers
Right: IgY and other major protein fractions recovered

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Product	Size	Total Sample Volume That Can Be Processed*	Item No.
Cleanascite™	10 ml	40 ml	X2555-10
Cleanascite™	50 ml	200 ml	X2555-50
Cleanascite™	100 ml	400 ml	X2555-100
Cleanascite™	1000 ml	4000 ml	X2555-1000

*Based on Cleanascite™ to Sample typical volume ratio. Volume ratio may be adjusted according to lipid levels.

Protocol

Supplied as an aqueous suspension of non-ionic adsorbent in saline, pH 8.0. When not in use, keep sealed. For best results store at 4°C. Do not freeze. **Cleanascite™** retains full activity when stored as directed for at least 6 months.

SAMPLE TYPE (partial list)	Volume Ratio, Cleanascite™ : Sample
General	1 : 5 to 1 : 1
Ascites Fluid	1 : 2 to 1 : 3
Serum, Fetal Calf Serum	1 : 2 to 1 : 3
Lipemic Serum	1 : 2 to 1 : 1
Egg Yolk suspension	1 : 1 to 2 : 1
Tissue homogenates	1 : 4 to 1 : 2

Actual lipid concentration in biological samples can vary greatly, so the ratios shown are only intended to provide general guidance in use.

1. Resuspend **Cleanascite™** by gently shaking. Excessive shaking or mixing will cause foaming. It should be completely resuspended prior to use.
2. Add 1 ml of **Cleanascite™** to 4 ml of the sample (or alternative ratio – see chart above). Mix the sample by gently shaking periodically for 10 minutes.
3. Centrifuge sample at 16,000 G's for 1-2 minutes - or - 2,000 – 3,000 G's for 15 minutes.
4. Decant supernatant containing macromolecules of interest and continue with purification, or analysis.

Optimization. Different sample volumes are easily scaled. Volume ratio can be adjusted up or down as required to remove the amount of lipids present**.**

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Key Reference Applications

Cell Response

Albakri, Marwah M., et al. "Fatty acids secreted from head and neck cancer induce M2-like Macrophages." Journal of Leukocyte Biology (2022).

To assess depletion of fatty acids from tumor supernatants, tumor-conditioned medium was treated with Cleanascite according to the manufacturer's instructions and prior to incubation with monocytes. The article states "**Depletion of Fatty acids with Cleanascite from FaDu or SCC supernatants largely reversed the phenotypic changes in Macrophage**s otherwise observed by incubating monocytes in these supernatants".

Yang, X. U. A. N., et al. " SCD1/FADS2 fatty acid desaturases equipoise lipid metabolic activity and redox-driven ferroptosis in ascites-derived ovarian cancer cells ." (2021).

The mechanisms underlying ovarian cancer (OvCa) cells dictating their lipid metabolic activities in promoting tumor progression remain elusive. The article states: "**Compared with the negative controls (OCM pretreated with the lipid removal reagent, Cleanascite)**, OvCa cells cocultured in the lipid-enriched OCM showed an increase of 18% in membrane fluidity."

Pointner, Lisa, et al. "Birch pollen induces Toll-like receptor 4-dependent dendritic cell activation favoring T cell responses." Frontiers in Allergy (2021): 42.

The article states "To remove the lipids in birch pollen extracts, Cleanascite™ was used …according to manufacturer's recommendations … in a ratio 1:1 (v/v). Importantly, **"non-specific treatment-associated and cytotoxic effects were ruled out …as neither the protein digestion nor the lipid extraction procedure affected cell activation."**

Wang, Xueyu, et al. "Epigenetic Silencing of miR-33b Promotes Peritoneal Metastases of Ovarian Cancer by Modulating the TAK1/FASN/CPT1A/NF-κB Axis." Cancers 13.19 (2021): 4795.

The effective use of Cleanascite™ helped establish that "… **depletion of fatty acids by Cleanascite in OCM significantly impaired ovarian cancer cell migration and invasion."**

Chen, Rain R., et al. "Targeting of lipid metabolism with a metabolic inhibitor cocktail eradicates peritoneal metastases in ovarian cancer cells." *Communications Biology* 2 (2019).

The article states: "To determine whether fatty acids in OCM are the primary energy source, fatty acids from OCM was first removed by Cleanascite™ Lipid Removal Reagent... Then, **XTT cell proliferation assays showed that the growth rate of ovarian cancer cells was remarkably reduced in cells cultured in Cleanascite™-treated OCM. Likewise, co-treatment with Cleanascite™ and OCM significantly attenuated the increased cell migration and invasion capacities of ES-2 and SKOV3 cells**.".

Lee, Hong-Jai, et al. "Regulatory effect of humoral milieu on the viral DNA and surface antigen expression of hepatitis B virus (HBV) in vitro." *Molecular & Cellular Toxicology* 15.2 (2019): 123-128.

The levels of HBsAg and HBV DNA were significantly decreased with lipid removal by Cleanascite™ in mouse serum rather than human serum".

Monoclonal Antibodies/Ascites

Collecting and Storing Hybridoma Tissue Culture Supernatants
doi:10.1101/pdb.prot103317 Cold Spring Harb Protoc 2020.

In the Troubleshooting section, the chapter states: "Problem (Step 7): A precipitate of lipids and/or cryoproteins has formed. Solution: This may be produced by long-term storage at 4°C. **These precipitates can be removed …using Cleanascite (Biotech Support Group; X2555) for clarification**."

Shapiro, Scott, et al. "Immunoglobulin G monoclonal antibodies to Cryptococcus neoformans protect mice deficient in complement component C3." *Infection and immunity* 70.5 (2002): 2598-2604.

"The ascites fluid was collected and centrifuged to remove cells. **Lipids and cell debris were removed with Cleanascite**."

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Extracellular vesicle clearance/cell response

Nguyen, Doan C., et al. "Extracellular vesicles from bone marrow-derived mesenchymal stromal cells support ex vivo survival of human antibody secreting cells." *Journal of extracellular vesicles* 7.1 (2018): 1463778.

Cleanascite™-treatment of the secretome dramatically reduced ASC functional survival, … Similar reductions were also noted with the secretome of non-irradiated MSC when treated with Cleanascite™ …"

Bile

Vesterhus, Mette, et al. "Novel serum and bile protein markers predict primary sclerosing cholangitis disease severity and prognosis." Journal of hepatology 66.6 (2017): 1214-1222.

Lukic, Natalija, et al. " An integrated approach for comparative proteomic analysis of human bile reveals overexpressed cancer-associated proteins in malignant biliary stenosis." *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1844.5 (2014): 1026-1033.

Egg Yolk

Ben Wade, Michelle Cummins, Anthony Keyburn and Tamsyn M. Crowley. Isolation and detection of microRNA from the egg of chickens. BMC Research Notes 2016 9:283.

Biofluids

Guyon, Léna, Anne-Claire Groo, and Aurélie Malzert-Fréon. "Relevant Physicochemical Methods to Functionalize, Purify, and Characterize Surface-Decorated Lipid-Based Nanocarriers." Molecular Pharmaceutics (2020).

"Conjugation of PPACK (a short chain peptide that inhibits thrombin) to liposomes was investigated through HPLC quantification of uncoupled peptide recovered from the supernatant after centrifugation of predialysis **PPACK-liposomes mixed with Cleanascite™ lipid adsorption reagent**. This indirect quantification was performed at a wavelength of 215 nm (detection of amide bond)."

Graeme T Clark, Paul J Russell, and Steven Westwood. Modification without impact: a case study in clinical assay failure due to lipemia. Bioanalysis; 2012: 4,(12):1421-1428

Organ Homogenates

Myerson, J., He, L., Lanza, G., Tollefsen, D. and Wickline, S. Thrombin-inhibiting perfluorocarbon nanoparticles provide a novel strategy for the treatment and magnetic resonance imaging of acute thrombosis. Journal of Thrombosis and Haemostasis.2011;9:1292-1300

Red Blood Cells

Antunes RF; Brandao C; Maia M; Arosa FA. Red blood cells release factors with growth and survival bioactivities for normal and leukemic T cells. Immunology and Cell Biology.2011;89(1):111-21

Tracheal Swab Samples

Li D, Wang J, Wang R, Li Y. A nanobeads amplified QCM immunosensor for the detection of avian influenza virus H5N1, Biosensors and Bioelectronics.2011;26(S10):4146-4154

Tissue and Cell Culture

Alhamdani MS, Schroder C, Hoheisel JD. Analysis conditions for proteomic profiling of mammalian tissue and cell extracts with antibody microarrays. Proteomics.2010;10(17):3203-7

Plasma/Serum

Dean, E. Danielle, et al. "Interrupted glucagon signaling reveals hepatic α cell axis and role for L-glutamine in α cell proliferation." *Cell metabolism* 25.6 (2017): 1362-1373.

The article states "For lipid removal, **whole mouse serum was treated with Cleanascite™ reagent (Biotech Support Group, Monmouth Junction, NJ) prior to islet culture at a 1:1 ratio according to the vendor's protocol. Lipid removal was validated by HPLC to remove 99% of all phopsholipids, cholesterols, and triglycerides**…."

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Taylor, Steven W., et al. "A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide." Clinica Chimica Acta (2016). **Cleanascite™ is shown both to improve LC-MS measurements, and validated in accordance with CLIA '88 guidelines.**

McIntyre, John A., et al. " Antiphospholipid autoantibodies as blood biomarkers for detection of early stage Alzheimer's disease." *Autoimmunity*0 (2015): 1-8.

Vaccine Research (Cholesterol Removal From Human Serum)

Kamtchoua, Thierry, Monica Bologa, Robert Hopfer, David Neveu, Branda Hu, Xiaohua Sheng, Nicolas Corde, Catherine Pouzet, Gloria Zimmerman, and Sanjay Gurunathan. Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults.Vaccine (2012).

Saliva

Lucy E. DesJardin Isolation of M. tuberculosis RNA from Sputum Methods in Molecular Medicine.2001;48:133-139

Patents

Shiffman, Dov, et al. "Methods for quantitation of insulin and c-peptide." U.S. Patent Application No. 15/942,188.

The application states "In some embodiments, serum is delipidated prior to quantitation by mass spectrometry. … In some embodiments, **the delipidation reagent is Cleanascite™**".

Yeast assays

Lifang, et al. Balanced globin protein expression and heme biosynthesis improve production of human hemoglobin in Saccharomyces cerevisiae. Metabolic Engineering (2013).

For a complete list of all Cleanascite™ references, visit:
http://www.biotechsupportgroup.com/References-s/138.htm#delipidation

CONTACT US

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