BIOTECH SUPPORT GROUP

# 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 and organ homogenates
- Clarifies saliva and fecal components
- Exquisite selectivity profile including extracellular vesicle and exosome clearance
- Extends the life of membrane and chromatographic columns.
- Enrichment of delipidated tissue samples
- For downstream processing of large-scale therapeutic proteins, enzymes and monoclonal antibodies.

**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.

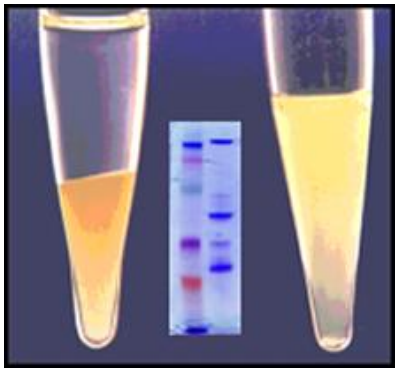
**Removes Lipid Factors**
**Phospho-Lipids**
**>99% Cholesterol &**
**Triglycerides**
**Lipoproteins**
**Extracellular Vesicles**
**(Exosomes)**

**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

1 Deer Park Drive, Suite M, Monmouth JCT, NJ 08852, USA • (P) 732-274-2866 • (F) 732-274-2899 •
www.biotechsupportgroup.com

BIOTECH SUPPORT GROUP

| 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 : 2 |
| Ascites Fluid | 1 : 4 |
| Serum | 1 : 4 |
| Egg Yolk suspension | 1 : 1 to 2 : 1 |
| Tissue homogenates | 1 : 4 to 1 : 2 |
| Transgenic Milk | 1 : 1 |

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. (1 : 4 volume ratio). Mix the sample by gently shaking periodically for 10 minutes.

3. Centrifuge sample at 16,000 G's for 1 minute - or - 1,000 G's for 15 minutes.

4. Decant supernatant containing macromolecules of interest and continue with purification.

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

BIOTECH SUPPORT GROUP

# Featured Cleanascite™ Reference Applications

**Cell Response**

Wang, Haiping, et al. "CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors." *Nature Immunology* (2020): 1-11.

Depleting regulatory T cells (Treg cells) to counteract immunosuppressive features of the tumor microenvironment (TME) is an attractive strategy for cancer treatment. However, systemic impairment of their suppressive function limits its therapeutic potential. Elucidating approaches that specifically disrupt intratumoral Treg cells is direly needed for cancer immunotherapy. The use of Cleanascite™ helped demonstrate that intratumoral Treg cells increase lipid metabolism and CD36 expression. The article states "cancer cell-conditioned medium … was treated with Cleanascite™ reagent (Biotech Support Group) before Treg cell culture at a volume ratio of 1:5 according to the manufacturer's instructions."

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).

Ovarian cancer is an intra-abdominal tumor in which the presence of ascites facilitates metastatic dissemination, and is associated with poor prognosis. However, the significance of metabolic alterations in ovarian cancer cells in the ascites microenvironment remains unclear. In this study, the authors investigated whether reprogramming of lipid metabolism in ovarian cancer cells could modulate cell viability and aggressiveness. 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 (Fig. 2d). Likewise, co-treatment with Cleanascite™ and OCM significantly attenuated the increased cell migration and invasion capacities of ES-2 and SKOV3 cells (Fig. 2e, f). These findings suggest that the fatty acid-enriched OCM provides as an energy source for supporting tumor growth and aggressiveness of ovarian cancer 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 investigations explored if humoral milieu such as serum or culture media, and its constituents, and pH would regulate the viral DNA and surface antigen expression of HBV *in vitro*. Furthermore, lipid removal analysis showed decreased level of HBV DNA and surface antigen expression in human and mouse serum. The article states "To evaluate the lipid exposure status within lipid bilayer, Cleanascite™ (Biotech Support Group) was added to HBV mixtures in the human serum, mouse serum, or DMEM, and the HBsAg and HBV DNA were evaluated. … we examined the virus-lipid interaction in non-host milieu, and compared the interaction between in host and non-host milieu. The levels of HBsAg and HBV DNA were significantly decreased with lipid removal by Cleanascite™ in mouse serum rather than human serum".

Sprenkle, Neil T., et al. "Endoplasmic reticulum stress is transmissible in vitro between cells of the central nervous system." *Journal of Neurochemistry*.

Improper protein folding and trafficking are common pathological events in neurodegenerative diseases that result in the toxic accumulation of misfolded proteins within the lumen of the endoplasmic reticulum (ER). The cell-extrinsic role of sustained unfolded protein response activation under physiological and pathological states in the central nervous system (CNS) remains to be elucidated. The authors studied the characteristics of a mediator secreted by ER stressed astrocytes and neurons. To determine if the mediator was a lipid associated factor, the article states "…100 µl of Cleanascite™ slurry was added to 1 ml of conditioned medium and incubated at RT with end-over-end mixing for 1 h followed by centrifugation."

BIOTECH SUPPORT GROUP

**Cell Response & Extracellular vesicle clearance**

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.

The study investigated the role of extracellular vesicles (EVs) in antibody secreting cell survival and IgG secretion. The article states "To understand whether disrupting the lipid plasma membrane that upsets the integrity of the EVs would compromise the survival activity of either the non-irradiated or irradiated secretomes, …We then cultured ASC with conventional media (vehicle), secretome from irradiated MSC, or secretome from irradiated MSC that had been pretreated with the lipid-disrupting agent Cleanascite™, which is known not to alter protein functionality [29]. 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™ … These results demonstrate that lipid-membrane bodies, such as EVs, could mediate important ASC survival factors within the MSC secretome."

**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.

Danese, Elisa, et al. " Assessment of bile and serum mucin5AC in cholangiocarcinoma: Diagnostic performance and biologic significance."*Surgery* (2014).

Farina, Annarita, et al. "Bile carcinoembryonic cell adhesion molecule 6 (CEAM6) as a biomarker of malignant biliary stenoses." *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* (2013).

Wang W, Ai KX, Yuan Z, Huang XY, Zhang HZ.Different Expression of S100A8 in Malignant and Benign Gallbladder Diseases.Digestive diseases and sciences. 2012.

Hauser-Davis RA, Lima AA, Ziolli RL, Campos RC.First-time report of metalloproteinases in fish bile and their potential as bioindicators regarding environmental contamination. Aquatic Toxicology.2012;110-111:99-106

Farina A, Dumonceau JM, Frossard JL. Proteomic Analysis of Human Bile from Malignant Biliary Stenosis Induced by Pancreatic Cancer Journal of Proteome Research.2009; 8(1):159-69

Chen Bo, Zheng Jian-wei, Wang Jian-ming, et al. Establishment and preliminary analysis of a 2-D human biliary map Chinese Journal of Hepatobiliary Surgery.2007

Chen B, Dong JQ, Chen YJ et al Two-dimensional electrophoresis for comparative proteomic analysis of human bile. Hepatobiliary & pancreatic diseases international.2007 Aug;6(4):402-6

Kristiansen TZ, Bunkenborg J, Gronborg M et al A Proteomic Analysis of Human Bile Molecular and Cellular Proteomics.2004;3:715-728

**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**

Farina, Annarita. "Pre-fractionation of Noncirculating Biological Fluids to Improve Discovery of Clinically Relevant Protein Biomarkers." *Proteomics for Biomarker Discovery*. Humana Press, New York, NY, 2019. 23-37.

For proteomic biomarker discovery, it is necessary to bridge the gap between basic and applied research by complying with clinical requirements. This chapter provides key suggestions for improving the discovery of clinically relevant protein biomarkers from body fluids. The chapter states : "If the elimination of lipids…is necessary, the sample can by treated with lipid removal (Cleanascite™)…

BIOTECH SUPPORT GROUP

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

Thakuria D, Schmidt O, Liliensiek AK. Field preservation and DNA extraction methods for intestinal microbial diversity analysis in earthworms. Journal of Microbiological Methods.2009;76(3):226-33

Cheng AM, Moore EE, Masuno T et al Normal Mesenteric Lymph Blunts the Pulmonary Inflammatory Response to Endotoxin. Journal of Surgical Research.2006;136(S2):166-171

McNally T, Mackie IJ, Machin SJ et al. Increased levels of beta 2 glycoprotein I antigen and beta 2 glycoprotein I binding antibodies are associated with a history of thromboembolic complications in patients with SLE and primary antiphospholipid syndrome British journal of rheumatology.1995 Nov;34(11):1031-6

**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

Fu LM, Shinnick TM. Genome-wide exploration of the drug action of capreomycin on Mycobacterium tuberculosis using Affymetrix oligonucleotide GeneChips Journal of Infection.2007;54(S3):277-284

Fu LM, Shinnick TM. Genome-wide analysis of intergenic regions of mycobacterium tuberculosis H37Rv using affymetrix genechips. EURASIP journal on bioinformatics & systems biology.2007:23054

**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

Czambel RK, Kharlamov A, Jones SC. Variations of brain endothelial nitric oxide synthase concentration in rat and mouse cortex.Nitric Oxide.2010;22(S1): 51-57

**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.

Decreasing glucagon action lowers blood glucose and may be useful therapeutically for diabetes. However, interrupted glucagon signaling leads to α cell proliferation. In this article, the authors wanted to determine which factors affected α cell proliferation. 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….".

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.

Palekar, Rohun U., et al. "Thrombin-Targeted Liposomes Establish A Sustained Localized Anticlotting Barrier Against Acute Thrombosis." Molecular pharmaceutics (2013).

Lijowski M, Caruthers S, Hu G. High-Resolution SPECT-CT/MR Molecular Imaging of Angiogenesis in the Vx2 Model Investigative Radiology.2009;44(1): 15–22

Turner JD, Langley RS, Johnston KL. Wolbachia Lipoprotein Stimulates Innate and Adaptive Immunity through Toll-like Receptors 2 and 6 to Induce Disease Manifestations of Filariasis The Journal of Biological Chemistry.2009;284:22364-22378

Torrelles JB, DesJardin LE, MacNeil J. et al Inactivation of Mycobacterium tuberculosis mannosyltransferase pimB reduces the cell wall lipoarabinomannan and lipomannan content and increases the rate of bacterial-induced human macrophage cell death Glycobiology.2009;19(7):743-755

Cho N, Chueh PJ, Kim C et al Monoclonal antibody to a cancer-specific and drug-responsive hydroquinone (NADH) oxidase from the sera of cancer patients. Cancer Immunology, Immunotherapy. 2002;51(3):121-9

Shapiro S, Beenhouwer DO, Feldmesser M et al. Immunoglobulin G Monoclonal Antibodies to Cryptococcus neoformans Protect Mice Deficient in Complement Component C3 Infect. Infection and immunity.2002;70(5):2598-604

Castro AR, Morrill We, Pope V. Lipid Removal from Human Serum Samples Clinical and diagnostic laboratory immunology.2000;7(2):197-199

**Saliva**

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

Beenhouwer DO, Shapiro S, Feldmesser M et al. Both Th1 and Th2 Cytokines Affect the Ability of Monoclonal Antibodies To Protect Mice against Cryptococcus neoformans Infection and immunity.2001;69: 6445-6455

Desjardin LE, Perkins MD, Wolski K et al. Measurement of Sputum Mycobacterium tuberculosis Messenger RNA as a Surrogate for Response to Chemotherapy American journal of respiratory and critical care medicine.1999;160(1):203-10

**Patents**

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

The inventors describe methods for diagnosing or prognosing insulin resistance in diabetic and pre-diabetic patients, the method comprising determining the amount of insulin and C-peptide in a sample. Mass spectrometric methods are described for detecting and quantifying insulin and C-peptide in a biological sample utilizing enrichment and/or purification methods coupled with tandem mass spectrometric or high resolution/high accuracy mass spectrometric techniques. The application states "In some embodiments, serum is delipidated prior to quantitation by mass spectrometry. … In some embodiments, the delipidation reagent is Cleanascite™".

Iwakura Yoichiro, Kakuta Shigeru, Suzuki, Shunsuke - United States Patent Application 20130011413.Method and Pharmaceutical Composition for Treatment of Intestinal Disease

Mcintyre, John A. United States Patent: 20120107841. Serum Diagnostic Method, Biomarker and Kit for Early Detection and Staging of Alzheimer's Disease

Morre, James D et al. United States Patent: 20030170757. Monoclonal antibodies specific for neoplasia-specific NADH: disulfide reductase

DJ Morre, NM McCarty, D Morre et al United States Patent: 7053188. Monoclonal antibodies specific for neoplasia-specific NADH: disulfide reductase

David C. Jones. United States Patent: 7999084. Devices and methods for reducing matrix effects

BIOTECH SUPPORT GROUP

**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).

**Yeast assays**

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

# CONTACT US

**We welcome your questions and comments regarding our products.**


Tel: 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-6pm EST.
Fax: 732-274-2899
Email: sales@biotechsupportgroup.com
Mail: 1 Deer Park Drive, Suite M, Monmouth Junction, NJ 08852
Web: www.biotechsupportgroup.com