BIOTECH SUPPORT GROUP
Sample Prep that Matters

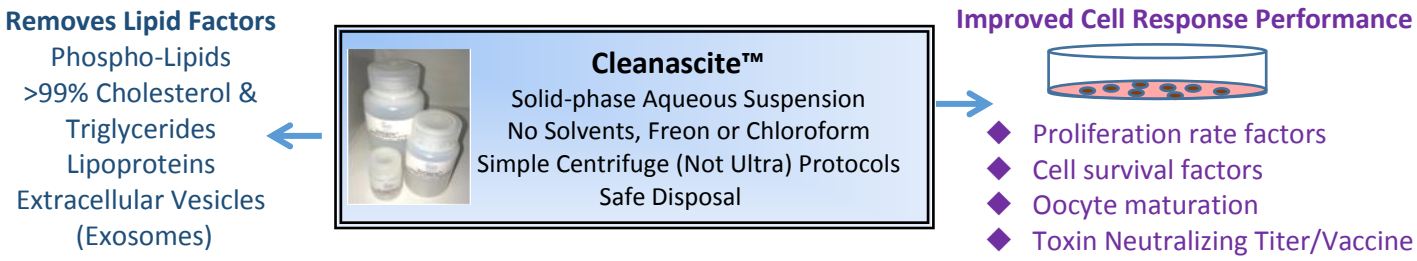
CLEANASCITE™
LIPID REMOVAL & CELL RESPONSE APPLICATIONS
AUGUST 6, 2019

Introduction

The "omics" revolution demanded new and different sample prep separations that were not efficiently performed by conventional technologies. While effective for many applications, these tools were not efficient for "omics" sample preparation, as throughput, economy and simplicity are especially required. Furthermore, these same separation tools often denatured proteins which limited there use in applications which demanded the measurement of function, structure or bio-activity.

For these reasons, BSG has been dedicated to create new methods and applications to drive efficient workflows and better data quality for all proteomic and biomarker analyses. Of special importance is the value created when certain families of biomolecules can be evaluated with respect to cell response and viability. For example, extracellular vesicles (EVs) substantially influence cultured cell behavior. While all of our products serve cell response applications, we report here an extensive list of applications in this area for **Cleanascite™**.

Cleanascite™ is derived through a proprietary formulation of metallic oxide derivatives. However, unlike other metallic oxides, **Cleanascite™** does not have significant protein binding, making its selectivity profile for lipids un-rivaled in the bio-research products industry. As a result, it is ideal to clear lipid-associated matrix effects - including extracellular vesicles, which may influence cell response assays.



Cleanascite™ has been validated in accordance with CLIA '88 guidelines. The applications and references for the many diverse investigations using **Cleanascite™** upstream of cell response measurements are next described.

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).

Cross-reference classifiers
Disease: Cancer
Sample Type: Conditioned media
Cell Response: Ovarian cancer cell lines

> 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.". The authors conclude that targeting the lipid metabolism signaling axis impedes ovarian cancer peritoneal metastases.

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.

Cross-reference classifiers
Disease: Hepatitis B
Sample Type: Serum
Cell Response: viral DNA and surface antigen expression

> 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". The authors' concluded that humoral lipid might confer protection to virion against toxicants or hostile interaction with humoral components.

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

Cross-reference classifiers
Disease: Neurodegenerative
Sample Type: Conditioned media
Cell Response: Stressed astrocytes and neurons

> 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." The authors provided evidence that depletion of lipids from astrocyte conditioned media using **Cleanascite** abrogated transmission of ER stress. Such evidence helped the authors conclude that ER stressed astrocytes and neurons secrete a molecule(s) with lipid characteristics which regulates both inflammatory and ER stress responses in other astrocytes, neurons, and microglia *in vitro*. These findings provide insight into the cell-nonautonomous influence of ER stress on cells of the central nervous system.

Barrera N, dos Santos Neto PC, Cuadro F, Bosolasco D, Mulet AP, Crispo M, et al. (2018) Impact of delipidated estrous sheep serum supplementation on in vitro maturation, cryotolerance and endoplasmic reticulum stress gene expression of sheep oocytes. PLoS ONE 13(6): e0198742. https://doi.org/10.1371/journal.pone.0198742

Cross-reference classifiers
Disease: Cryosurvival
Sample Type: Estrous sheep serum
Cell Response: Oocyte maturation

> High lipid content of oocytes and embryos in domestic animals is one of the well-known factors associated with poor cryosurvival. In this articles, the authors wanted to determine whether the use of delipidated estrous sheep serum during in vitro maturation (IVM) of ovine oocytes reduces the cytoplasmic lipid droplets content and improves embryo development and cryotolerance after vitrification. The article states "Lipid removal from serum was performed by using **Cleanascite™** (Biotech Support Group, NJ, USA) according to the instructions provided by the manufacturer. Unlike other approaches, the protocol described herein for delipidation of estrous sheep serum was effective in decreasing levels of Triglycerides, total Cholesterol, and NEFAs. To our knowledge this is the first study to use the **Cleanascite™** method to generate estrous sheep serum yielding significantly reduced lipid levels. Subsequent use of the partially delipidated serum as supplemented in IVM media resulted in effective reduction of oocyte lipid content. The advantage of this method over other traditional methods (i.e. chloroform) includes increased feasibility and reduced toxicity and biosafety concerns". Their results demonstrate that although supplementation of IVM medium with delipidated estrous sheep serum reduces the presence of cytoplasmic lipid droplets in oocytes after maturation, oocyte cryotolerance is not improved.

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.

Cross-reference classifiers
Disease: Pan/Methods
Sample Type: Bone marrow-derived mesenchymal stromal cell secretome
Cell Response: Human antibody secreting cells

> Bone marrow-derived mesenchymal stromal cells (MSC) have been shown to support human antibody secreting cells (ASC) survival ex vivo. Extracellular vesicles from bone marrow-derived mesenchymal stromal have novel mechanisms of cell-cell communication over short and long distances, but whether the crosstalk between these cell interactions can occur via extracellular vesicles is not known. Thus, 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** [28], 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."

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.

Cross-reference classifiers
Disease: Diabetes
Sample Type: Mouse serum
Cell Response: α cell proliferation

> 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 phospholipids, cholesterols, and triglycerides….". In testing whether lipids could stimulate α cell proliferation, it was found that serum activity was retained after the removal of >99% of triglycerides, cholesterols, and phospholipids. The authors conclude that amino acids, especially L-glutamine, regulate α cell proliferation and mass via mTOR-dependent nutrient sensing.

Lovászi, M., et al. "Sebum lipids influence macrophage polarization and activation." *British Journal of Dermatology* (2017). doi: 10.1111/bjd.15754.

Cross-reference classifiers
Disease: Cell Biology
Sample Type: Sebocytes
Downstream Use Platform: Immortalized human SZ95 sebocytes

> The article's authors report on sebum lipids contributing to the differentiation, polarization and function of macrophages. In order to determine the role of specific lipids, lipid removal was investigated from supernatants of the immortalized human SZ95 sebocytes, as stated, "For lipid depletion of the supernatants **Cleanascite** lipid clarification reagent (Biotech Support Group, Monmouth Junction, NJ, USA) was used according to the manufacturer's instructions. Lipids; squalene, linoleic acid, oleic acid, palmitic acid and stearic acid (Sigma-Aldrich); were replaced individually subsequent to lipid depletion in a concentration of 150 μM.". The authors concluded a role for sebaceous glands in modulating immune responses via their secreted lipids that are of possible pathologic and therapeutic relevance.

Chan, DW, Mak, SL, Ngan, HYS. The significance of lipid metabolism in peritoneal metastases of ovarian cancer. The 2016 Cold Spring Harbour Asia Conference on Cancer and Metabolism, Suzhou, China, 19-23 September 2016. http://hub.hku.hk/handle/10722/235385

Cross-reference classifiers
Disease: Cancer
Sample Type: Omental explant culture
Downstream Use Platform: Ovarian oncogenic capacities

> The authors report that the high lipid content in ascetic fluid provides a huge energy source for ovarian cancer cells in peritoneal dissemination and intraperitoneal tumor colonization. In this study, ovarian cancer cells co-cultured with an omental explant culture system (OCM) or ascetic fluid from ovarian cancer patients exhibited an increase in *in vitro* cell growth, cell migration/invasion through activation of TAK1/NF-kappaB signaling cascade. The abstract states "In contrast, the oncogenic capacities of ovarian cancer cells were impaired when cultured in OCM treated with **Cleanascite** Lipid Removal Reagent, suggesting that the bioactive lipids in OCM are required for enhanced oncogenic capacities".

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).

Cross-reference classifiers
Disease: Infectious Disease Vaccine
Sample Type: Serum
Downstream Use Platform: toxin-neutralizing antibody titer

> Authors Thierry Kamtchoua et al published an article in the journal Vaccine titled, 'Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults' describing the immunogenicity of pneumococcal single antigen protein vaccine in a phase 1, randomized, placebo controlled dose escalating study. Authors cite **Cleanascite™** from Biotech Support Group for removal of cholesterol from serum. A toxin neutralizing assay with antibodies in sera was developed to neutralize cytotoxicity caused by Ply in Vero cells. An incubated challenge dose of pneumolysin toxin containing serum diluted with or without **Cleanascite™** was developed. The neutralizing titer inhibited the toxin's effect on Vero cells. According to the paper, "Briefly, the toxin-neutralizing antibody titer was determined by incubating a challenge dose of pneumolysin toxin with serial 2-fold dilutions of serum treated with or without **Cleanascite™** (Biotech Support Group) to remove cholesterol, an inhibitor of Ply".

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

Cross-reference classifiers
Disease: Cancer
Sample Type: Red Blood Cell media
Downstream Use Platform: T Cell proliferation

Red blood cells (RBCs) have been implicated since the early 1970s in the modulation of T cell responses both *in vitro* and *in vivo*. As they can also regulate biological processes of neighboring cells, the authors aim was to show that human red blood cell conditioned media contains bioactive factors that favor proliferation of normal activated T cells and leukemic Jurkat T cells. To define whether factors that favor proliferation were lipid associated, the RBC media was depleted of extracellular vesicles by ultracentrifugation. Then, the article states "For lipid depletion, **Cleanascite™** was added to the RBC-sup in a ratio 1:4 and the mixture incubated first in a rotator at room temperature for 10 min, followed by a further incubation at 4ºC for 30 min, following manufacturer's instructions. Then, the mixture was centrifuged to remove the resin and the RBC-sup collected and concentrated as indicated above before the *in vitro* bioactivity assays". The authors conclude that red blood cells release protein factors (not lipid factors) with the capacity to sustain T-cell growth and survival. Such protein factors may have an unforeseen role in sustaining malignant cell growth and survival *in vivo*.

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

Cross-reference classifiers
Disease: Infectious Disease
Sample Type: Soluble *Brugia malayi* female worm extract
Downstream Use Platform: HEK-TLR2 cells

Wolbachia endosymbiotic bacteria have been implicated in the inflammatory pathogenesis of filariasis. Inflammation induced by *Brugia malayi* female worm extract (BMFE) is dependent on Toll-like receptors 2 and 6 (TLR2/6) with only a partial requirement for TLR1. Removal of *Wolbachia*, lipids, or proteins eliminates all inflammatory activity. The article states "To determine if TLR2/6 ligands of *Wolbachia* are lipoproteins, we treated the filarial extracts with **Cleanascite™**, which selectively removes lipids and lipoproteins, or with **BindPro™**, a polymeric protein removal suspension reagent (Biotech Support Group). Both treatments completely ablated (to background levels) HEK-TLR2 cell IL-8 reporter gene activity to BMFE (Fig. 1*C*) thereby showing that the TLR2/6 activity depends on both lipid and protein moieties." The authors conclude that *Wolbachia* lipoproteins drive interferon-dependent $CD4^+$ T cell polarization and antibody switching.

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

Cross-reference classifiers
Disease: Hemorrhagic shock
Sample Type: Mesenteric lymph
Downstream Use Platform: Primary human pulmonary endothelial cells

LPS induced ICAM-1 expression decreases by lipoproteins in normal mesenteric lymph(NML) which contain anti-inflammatory factors**. Cleanascite™** was used for delipidation and removal of lipoproteins from primary human pulmonary endothelial cells (HMVECs) incubated with normal mesenteric lymph NML or post-shock mesenteric lymph PSML. ICAM expression was measured after LPS stimulation by flow cytometry. ICAM-1 surface expression was measured by flow cytometry. **Cleanascite™** extracted lipoproteins from NML before incubation and LPS-induced ICAM-1 expression was determined. Researchers concluded that decreased lipoprotein expression after hemorrhagic shock HS increases post-shock mesenteric lymph PSML toxicity from the ischemic gut.

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

Cross-reference classifiers
Disease: Cancer
Sample Type: Serum
Downstream Use Platform: Cancer cell cultures

Scientists prepared monoclonal antibodies to a 34-kDa circulating form of a drug-responsive hydroquinone (NADH) oxidase with a protein disulfide–thiol interchange activity specific to the surface of cancer cells and the sera of cancer patients**. Cleanascite™** was used for deplipidation of sera. Epitopes (antibody (mAb) 12.1 and postimmune antisera) inhibited the drug-responsive oxidation of NADH with the sera of cancer patients. Authors

concluded both mouse ascites containing mAb 12.1 and postimmune sera (but not preimmune sera) slowed the growth of human cancer cell lines in culture, but did not affect the growth of non-cancerous cell lines.

Patents (Third Party)

United States Patent Application 20170348398 entitled: "COMPOSITIONS AND METHODS FOR DECREASING BLOOD GLUCAGON LEVELS"

Cross-reference classifiers
Disease: Diabetes
Sample Type: Serum
Cell Response: Pancreatic α cell proliferation

The patent discloses compositions and methods for decreasing blood glucagon levels. As disclosed, L-glutamine is a selective stimulator of α-cell proliferation generated when glucagon signaling is interrupted. A method for treating a subject with hyperglucagonemia, e.g., a subject with diabetes, that involves administering to the subject a composition comprising an L-glutamine inhibitor in an amount effective to decrease blood glucagon levels, is disclosed. In an example, pancreatic islets were isolated from male 8-14 week old C57B16/J mice (Jackson Laboratory, ME) and cultured in various media conditions for 3 days. The patent states " For lipid removal, serum was treated with **Cleanascite** reagent (Biotech Support Group, Monmouth Junction, N.J.) prior to islet culture at a 1:1 ratio according to the vendor's protocol. ". The example supports that increased amino acids, but not lipids and other soluble factors, selectively increased rapamycin-sensitive α-cell proliferation.

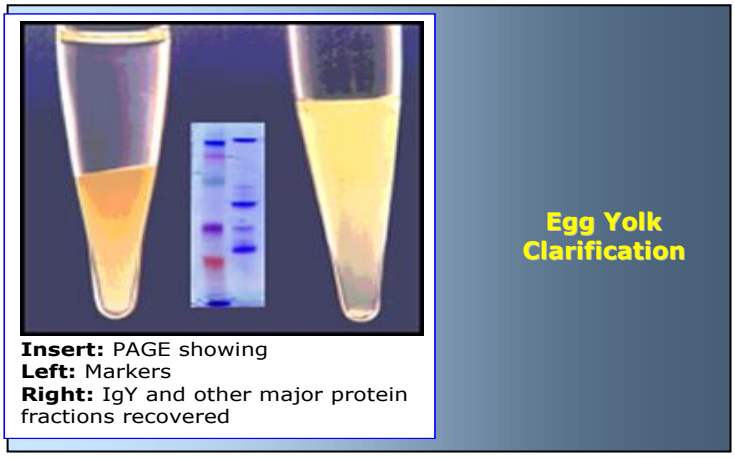
Lipid Removal & Clarification

• Extensively cited • Replaces hazardous hydrocarbons • Diverse samples

Cleanascite™

Lipid Adsorption & Clarification

- 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.



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

Cleanascite™ is derived through a proprietary formulation of metallic oxide derivatives. Unlike other metallic oxides, **Cleanascite™** does not have significant protein binding making its selectivity profile for lipids unique in the bio-research products industry. As a result, it is ideal to clear lipid-associated matrix effects from human sera, bile, ascites, and other high lipid content sample types.

Discard
Lipids
Cholesterol
Extracellular Vesicles

Cleanascite™
Solid-phase Aqueous Suspension
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

Cleanascite™ is supplied as a suspension reagent. Simply add, mix and centrifuge in a 10 minute protocol.

Key References

Plasma/Serum Protein Biomarkers

The authors aimed at simultaneously measuring intact insulin and proinsulin derived C-peptide, to help predict development of diabetes mellitus, as well as in differential diagnosis in cases of hypoglycemia. **Cleanascite™** is shown both to improve LC-MS measurements, and validated in accordance with CLIA '88 guidelines. { doi: 10.1016/j.cca.2016.01.019 }

Vaccine Development

To evaluate immunogenic response to a vaccine candidate, it is necessary to measure the antibodies from sera; a sample with a diverse lipid profile. In this citation, **Cleanascite™** was used in a toxin neutralizing assay to evaluate the influence of cholesterol dependency, on a candidate protein pneumococcal vaccine. { doi: 10.1016/j.vaccine.2012.11.005 }

Bile Proteomics

The authors report methods to overcome the biological variability of analyzing a high number of bile samples. They concluded that delipidation yielded a considerable number of complementary protein identifications and that **Cleanascite**™ treatment was indispensable for in-solution digestion methods. { http://dx.doi.org/10.1016/j.jprot.2016.11.021 }

Ascites Monoclonal Antibodies

The researchers determined the role of complement on MAb-mediated protection for four mice Ig subclasses. After centrifugation of ascetic fluid, **Cleanascite™** protocol was implemented to remove lipids. { doi: 10.1128/IAI.70.5.2598-2604.2002 }