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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
- Related 'LX' product alternative to LipoClear, go to: https://www.biotechsupportgroup.com/Cleanascite-LX-p/lx155.htm

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.

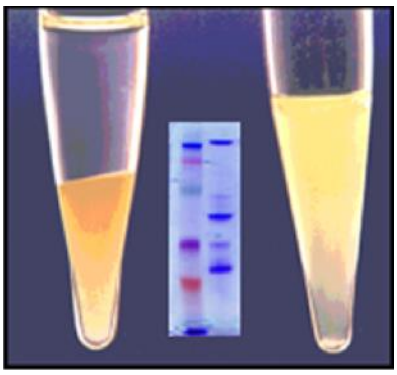
Removes Lipid Factors
Phospho-Lipids
>99% Cholesterol &
Triglycerides
Lipoproteins
Extracellular Vesicles
(Exosomes)

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 : 4
Serum	1 : 4
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 minute - or - 1,000 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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Cleanascite™ 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).

Tumor-infiltrating monocytes can mature into Macrophages that support tumor survival or that display antitumor properties. To explore mechanisms steering Macrophage maturation, the authors assessed the effects of supernatants from squamous cell carcinoma cell lines (FaDu and SCC) on monocyte-derived Macrophage maturation. 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". Macrophages incubated with either palmitic acid or oleic acid developed similar phenotypes as cells incubated in tumor supernatants. The authors conclude that fatty acids are sufficient to mediate monocyte skewing towards M2-like cells.

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).

Malignant ascites in peritoneal metastases is a lipid-enriched microenvironment and is frequently involved in the poor prognosis of epithelial ovarian cancer (EOC). However, the detailed mechanisms underlying ovarian cancer (OvCa) cells dictating their lipid metabolic activities in promoting tumor progression remain elusive. For this, the BSG product Cleanascite™ was used as a lipid-cleared control in the investigation. 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." The authors describe a disruption of the cellular redox balance and subsequent iron-mediated lipid peroxidation in ascites-derived OvCa cells.

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

This study aimed to examine (i) the importance of Toll-like receptor 4 (TLR4) for dendritic cell (DC) activation by birch pollen extracts (BPE), (ii) the extent of the contribution of BPE-derived lipopolysaccharide (LPS) and other potential TLR4 adjuvant(s) in BPE, and (iii) the relevance of the TLR4-dependent activation of BPE-stimulated DCs in the initiation of an adaptive immune response. The article states "To remove the lipids in BPE, CleanasciteTM 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."** These findings suggest that TLR4 is a major pathway by which BPE triggers DC activation that is involved in the initiation of adaptive immune responses.

Flori, E., Mastrofrancesco, A., Mosca, S., Ottaviani, M., Briganti, S., Cardinali, G., Filoni, A., Cameli, N., Zaccarini, M., Zouboulis, C.C., Picardo, M., Sebocytes contribute to melasma onset, ISCIENCE (2022), doi: https://doi.org/10.1016/j.isci.2022.103871.

Melasma is a hyper-pigmentary disorder with photoaging features, whose manifestations appear on specific face areas, rich in sebaceous glands. To verify whether sebocyte-specific lipids could contribute to the observed changes in cell lines, the investigators tested lipid depleted irradiated SZ95 conditioned medium to treat these cells. The article states: "Cleanascite was added to the collected SZ95 medium in a ratio 1:4 and mixed for 10 min at room temperature by gentle shaking. Following centrifugation (16000g) for 1 min at 4 °C, the supernatant was carefully decanted into a clean collection vial and used for experiments." The authors indicate

sebocytes as one of the actors in melasma pathogenesis, inducing prolonged skin cells stimulation, contributing to localized dermal aging and hyperpigmentation.

Di Conza, Giusy, et al. "Tumor-induced reshuffling of lipid composition on the endoplasmic reticulum membrane sustains macrophage survival and pro-tumorigenic activity." Nature immunology (2021): 1-13.

Tumor-associated macrophages (TAMs) display pro-tumorigenic phenotypes for supporting tumor progression in response to microenvironmental cues imposed by tumor and stromal cells. However, the underlying mechanisms by which tumor cells instruct TAM behavior remain elusive. The investigators here uncover that tumor-cell-derived glucosylceramide stimulated unconventional endoplasmic reticulum (ER) stress responses by inducing reshuffling of lipid composition and saturation on the ER membrane in macrophages. As part of the study, a comparison of culture media (CM) with and without lipids (woLip) was made. The article states "To generate culture media without lipids, …CM was treated with Cleanascite according to the manufacturer's instructions.". The authors uncover the unexpected roles of tumor-cell-produced lipids that simultaneously orchestrate macrophage polarization and survival in tumors.

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 tumor microenvironment is known to influence cancer epigenomics, which plays an essential role in promoting tumor development and metastatic progression. To study this, the researchers used ovarian cancer cells cocultured in omental conditioned medium (OCM), which mimics the ascites microenvironment, and in vivo tumor growth. To examine whether fatty acids in OCM are the main source of energy for tumors, the article states "The omental mixture was subsequently added to the cell culture medium supplemented with 1% FBS for 24 h. OCM was filtered and stored at 4 °C prior to the removal of omentum tissues by centrifugation. To selectively remove lipids and cell debris, Cleanascite™ … was employed for OCM according to the manufacturer's suggestions." XTT cell viability analysis was performed and showed that the cell growth rate of ES-2 and MES-OV cells was remarkably reduced when cocultured in lipid-depleted OCM. The effective use of Cleanascite™ helped establish that "both miR-33b overexpression and **depletion of fatty acids by Cleanascite in OCM significantly impaired ovarian cancer cell migration and invasion."**

Chen, Hsin-Yi, et al. "Selective abrogation of S6K2 maps lipid homeostasis as a survival vulnerability in MAPKi-resistant NRASmut melanoma." bioRxiv (2021).

The article reports that silencing of the ribosomal protein S6 kinase 2 (S6K2), while preserving the activity of S6K1, perturbs lipid metabolism, enhances fatty acid unsaturation, triggers lipid peroxidation and induces cell death selectively in NRAS-mutant melanoma cells that are resistant to MAPK inhibition. To help identify cell response factors, the article states "Lipid-depleted serum (FBS) was prepared by treating with Cleanascite reagent at a volume ratio of 1:4 per the manufacturer's instructions." The study establishes S6K2 and its effector subnetwork as promising targets for NRAS-mutant melanoma that are resistant to global MAPK pathway inhibitors.

"Miller-Rhodes, Patrick, and Harris A. Gelbard. The Cell Culture Environment Regulates the Transcription Factor MafB in BV-2 Microglia. " Matters 7.1 (2021): e202010000001.

Microglia experience dramatic molecular and functional changes when transferred from the central nervous system (CNS) to a cell culture environment. This investigation explores lipid dependency as CNS-specific microenvironmental cues, that dictate the gene-regulatory networks specified by master regulator transcription factors such as V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB). To support this analysis, the authors evaluated serum lipid-depletion, stating "Lipids were depleted from FBS using a Cleanascite lipid removal agent (Biotech Support Group, cat no. X2555-10). Cleanascite reagent was thoroughly resuspended before mixing with FBS at a volume ratio of 1:4 (Cleanascite: FBS)." The report concludes that depletion of lipids, either by serum deprivation or the use of lipid-depleted serum, reduced MafB protein levels in BV-2

microglial cells. In aggregate, the data suggest that serum exposure regulates the transcription factor MafB in BV-2 cells through direct and indirect mechanisms.

Gomes, Ana P., et al. "Age-induced accumulation of methylmalonic acid promotes tumour progression." Nature (2020): 1-5. https://doi.org/10.1038/s41586-020-2630-0

The risk of cancer and associated mortality increases substantially in humans from the age of 65 years onwards. The authors describe how metabolic alterations that occur with age can produce a systemic environment that favors the progression of tumors. Specifically, that methylmalonic acid (MMA), a by-product of propionate metabolism, is upregulated in the serum of older people and functions as a mediator of tumor progression. To support this analysis, the authors state "HS (human serum) samples were manipulated to assess the components … that might facilitate entrance of MMA into cells. To delipidate the HS, Cleanascite Lipid Removal Reagent (Biotech Support Group) was used according to the manufacturer's protocol …, using a 1:4 volume ratio of Cleanascite reagent to sample".

Stahl, Elia, et al. "Phosphatidylcholines from Pieris brassicae eggs activate an immune response in Arabidopsis." eLife 9 (2020): e60293.

Recognition of conserved microbial molecules activates immune responses in plants, a process termed pattern-triggered immunity (PTI). Similarly, insect eggs trigger defenses that impede egg development or attract predators, but information on the nature of egg-associated elicitors is scarce. Preliminary tests indicated that egg-derived defense eliciting compounds are of lipidic nature. To confirm this, the authors stated "we used Cleanascite solid-phase aqueous reagent to selectively adsorb lipids from EE (Egg Extract). Application of the lipid-containing phase to …*Arabidopsis* reporter lines triggered strong and localized GUS staining, similar to EE treatment. In contrast, the supernatant containing proteins and other non-lipidic molecules was not active, indicating that defense gene-inducing molecules were restricted to the lipid phase (Figure 1A)". This helped the research in the identification of phosphatidylcholines (PCs) as egg-associated molecular patterns (EAMPs), and illustrated the acute ability of plants to detect conserved immunogenic patterns from their enemies.

Chan DW, Yung MM, Chan Y-Sang, Xuan Y, Yang H, Xu D, Zhan J-Biao, Chan KK, Ng T-Bun, Ngan HY, MAP30 protein from Momordica charantia is therapeutic and has synergic activity with cisplatin against ovarian cancer in vivo by altering metabolism and inducing ferroptosis, Pharmacological Research (2020), doi: https://doi.org/10.1016/j.phrs.2020.105157

Increasing evidence shows that Traditional Chinese Medicine has an obvious appeal for cancer treatment, but there is still a lack of scientific investigation of its underlying molecular mechanisms. The investigators report that a bioactive protein, MAP30, isolated from bitter melon seeds exhibited potent anticancer and anti-chemoresistant effects on ovarian cancer cells. To support the analysis of MAP30 altering glycolysis and lipid metabolism in ovarian cancer cells, the authors state "Nile Red fluorescence of lipid-loaded ES2 (human epithelial ovarian cancer cells) in OCM culture for 48 h. …OCM (Omental conditioned medium) with Cleanascite™ treatment were used as negative controls.". The authors conclude that natural MAP30 is a non-toxic supplement that may enhance chemotherapeutic outcomes and benefit ovarian cancer patients with peritoneal metastases.

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."

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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. Likewise, co-treatment with Cleanascite™ and OCM significantly attenuated the increased cell migration and invasion capacities of ES-2 and SKOV3 cells**. 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."

Monoclonal Antibodies/Ascites

Greenfield, Edward A. "Inducing, collecting, and storing ascites." Cold Spring Harbor Protocols 2021.10 (2021): pdb-prot103309.

Ascitic fluid (also called ascites) is an intraperitoneal fluid extracted from mice that have developed a peritoneal tumor. For antibody production, the tumor is induced by injecting hybridoma cells into the peritoneum, which serves as a growth chamber for the cells. The hybridoma cells grow to high densities and continue to secrete the antibody of interest, thus creating a high-titered solution of antibodies for collection. In the TROUBLESHOOTING section, the book states:
Problem (Step 8): 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 by centrifugation or by using Cleanascite (Biotech Support Group X2555) for clarification.

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

For most immunochemical methods, tissue culture supernatants will be the most useful source of monoclonal antibodies. The supernatants are not contaminated with high levels of other antibodies, and the concentration is

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high enough for most assays if used undiluted. Procedures for collecting tissue culture supernatants are described. 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."

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).

Malignant ascites in peritoneal metastases is a lipid-enriched microenvironment and is frequently involved in the poor prognosis of epithelial ovarian cancer (EOC). However, the detailed mechanisms underlying ovarian cancer (OvCa) cells dictating their lipid metabolic activities in promoting tumor progression remain elusive. For this, the BSG product Cleanascite™ was used as a lipid-cleared control in the investigation. 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." The authors describe a disruption of the cellular redox balance and subsequent iron-mediated lipid peroxidation in ascites-derived OvCa cells.

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."

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.

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

BIOTECH SUPPORT GROUP

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™)…

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).

Surface functionalization of lipid-based nanocarriers (LBNCs) with targeting ligands has attracted huge interest in the field of nanomedicines for their ability to overcome some physiological barriers and their potential to deliver an active molecule to a specific target without causing damage to healthy tissues. In this review article, the authors state the use of Cleanascite™ in the analysis of LBNCs; “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

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

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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

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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

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

Yeast assays

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

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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