CLEANASCITE™

LIPID REMOVAL & CLARIFICATION REFERENCE APPLICATIONS

JANUARY 16, 2017

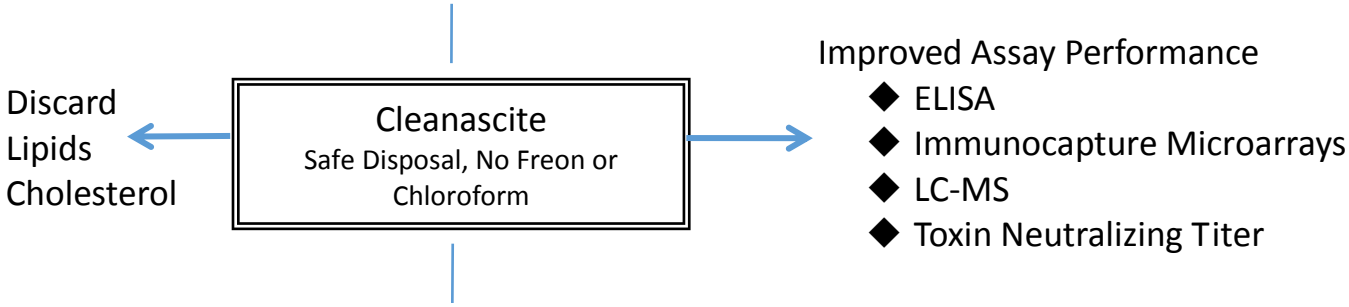
BIOTECH SUPPORT GROUP

Sample Prep that Matters

Introduction

The "omics" revolution demanded new and different sample prep separations that were not efficiently performed by conventional technologies. For years the protein separations toolkit was limited to liquid chromatography and gel electrophoresis. While effective for many applications, such tools were not efficient for "omics" sample preparation, when throughput, economy and simplicity were required. Furthermore, these same separation tools most often denatured proteins which limited there use in applications which required the measurement of function, structure or bio-activity.

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.



The applications and references for use of **Cleanascite™** follows.

Plasma/Serum

Steven W. Taylor, Nigel J. Clarke, , Zhaohui Chen, Michael J. McPhaul. A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide. Clinica Chimica Acta
Available online 25 January 2016. doi:10.1016/j.cca.2016.01.019

In brief, the article's 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. The article states "…15 µl of internal standard were added to each well followed by 50 µl of Cleanascite™ delipidation reagent previously mixed into a uniform suspension by a brief aspiration/dispense cycle within its reagent reservoir." The article further notes a key component of the methodology as "…the use of a delipidation reagent to enhance immunocapture…The result was greatly enhanced recoveries and tighter CVs for the IS {internal standard} throughout the plate".

"This is an exciting development as **Cleanascite™** is shown both to improve LC-MS measurements, and validated in accordance with CLIA '88 guidelines. Clearly, there was necessity for removing lipids without compromising the quantity or quality of the 2 biomarkers present and to be measured. The exquisite selectivity profile of **Cleanascite**™ makes this possible." states Swapan Roy, Ph.D., President and Founder of Biotech Support Group.

Knight, Jason S., Wei Luo, Alexander A. O'Dell, Srilakshmi Yalavarthi, Wenpu Zhao, Venkataraman Subramanian, Chiao Guo et al. "Peptidylarginine Deiminase Inhibition Reduces Vascular Damage and Modulates Innate Immune Responses in Murine Models of Atherosclerosis." *Circulation research* (2014): CIRCRESAHA-113.

Neutrophil extracellular trap (NET) formation leads to thrombosis and blocking peptidylarginine deiminase (PAD) with Cl-amidine reduces atherosclerosis. NET formation is a marker for sepsis, cancer, thrombosis, autoimmune disease. Authors Knight et al published an article in the journal Clinical Research which cites **Cleanascite™** from Biotech Support Group for lipid clarification and adsorption from serum samples. The article quotes, "Clearance of lipids from serum. Lipids were removed by **Cleanascite** Lipid Removal Reagent (Biotech Support Group, Monmouth Junction,NJ) according to manufacturer's instructions. The protocol removed >80% of total cholesterol and triglycerides."

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

For the preparation of nanoparticle molecular imaging agent that affords sensitive nuclear detection in conjunction with high-resolution MR characterization of tumor angiogenesis. Molecular imaging allows researchers to study integrins on proliferating endothelial cells during angiogenesis. Scientists combined 99mTc imaging and MRI to provide high sensitivity detection with high-resolution 3D neovasculature. Comixture of the integrin-targeted 99mTc nanoparticles included 3 mole% bis-pyridyl-lysine-caproyl-phosphatidylethanolamine, 0.1 mole% vitronectin antagonist complexed to PEG2000-phosphatidylethanolamine, and high purity egg phosphatidylcholine for balance. The surfactant comixture of the integrin-targeted particles 99mTc-gadolinium nanoparticles included 30 mole% gadolinium diethylene-triamine-pentaacetic acid-bis-oleate as an equimolar substitution for the lecithin. During the preparation of 99mTc-Tricarbonyl precursor and 99mTc nanoparticles **Cleanascite™** (300 µL) was added to the reaction mixture to precipitate the nanoparticles. The nanoparticles retained 97% of the 99mTc in plasma.

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

In this article, researchers used **Cleanascite™** first to determine if TLR2/6 ligands of Wolbachia are lipoproteins for removing lipids and lipoproteins. Next **BindPro™**, a polymeric protein removal suspension reagent (Biotech Support Group) was used to ablate levels of HEK-TLR2 cell IL-8 reporter gene activity to BMFE thereby showing that the TLR2/6 activity depends on both lipid and protein moieties.

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

Authors reviewed the efficacy of lipid removal containing antibodies to treponemal and nontreponemal syphilis antigens from human serum samples by using **Cleanascite™** compared to a reference chloroform method. The lipid content was measured before and after treatment. Amount of lipid removal ranged from 61 to 70% with **Cleanascite™** and 60 to 62% with chloroform. Moreover, authors praisde **Cleanascite™** for being more environmentally friendly than chloroform.

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

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.

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

The effect of complement component C3 on mice affected by Cryptococcus neoformans was studied by researchers to determine the role of complement on Ab-mediated protection for four mice Ig subclasses (IgG1), IgG2a, IgG2b, IgG3 switch variants. Role of complement component C3 in Ab-mediated protection was determined by passive administration of MABs and reviewing the course of disease progression. Ascitic fluid was obtained by intraperitoneal (i.p.) injection of hybridoma cells into SCID mice. After centrifugation of ascetic fluid, **Cleanascite™** protocol was implemented to remove lipids and cell debris. ELISA quantified the Ab concentration. Results showed IgG MAbs protect against cryptococcal infection in mice in the absence of C3.

Vaccine research relies on the systemic immunogenic response to the vaccine candidate. To evaluate such a response, it is necessary to measure the antibodies from sera; a sample type with a diverse lipid profile between individuals. Because lipids can often impact antibody analysis by specific and non-specific matrix effects, for vaccine development, it is beneficial to deplete lipids prior to analysis. The Biotech Support Group product – **Cleanascite**™ has the necessary selectivity profile to support this demanding application.

In this citation, **Cleanascite™** was used in a toxin neutralizing assay to evaluate the influence of cholesterol dependency, on a candidate protein pneumococcal vaccine.

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).

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".

Nussbaum G, Cleare W, Casadevall A et al Epitope Location in the Cryptococcus neoformans Capsule Is a Determinant of Antibody Efficacy The Journal of experimental medicine.1997;185:685-694

For the preparation of monoclonal antibodies from ascites of hybridoma cells, **Cleanascite™** was used for lipid removal. Monoclonal antibodies (mAbs) to the polysaccharide capsule of Cryptococcus neoformans can prolong survival in mice. epitope specificity in determining protective efficacy was suggested by experiments with two murine IgM anticryptococcal mAbs, 12A1 and 13F1. The protective mAb, 12A1, produced a homogeneous annular fluorescence pattern, whereas the nonprotective mAb, 13F1, produced a punctate pattern of fluorescence on one strain of serotype D, C. neoformans.

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

The article describes localized site targeted treatment of acute thrombosis in which D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK)-liposomes. The article cites **Cleanascite™** "quantification of uncoupled PPACK recovered from the supernatant after centrifugation of pre-dialysis PPACK-Liposomes mixed with Cleanascite lipid adsorption reagent (Biotech Support Group, Monmouth, NJ). As opposed to heparin which does not bind to clot-bound thrombin as the heparin binding site is hidden, PPACK-liposomes attracts pro-coagulants at the new clot site. Predialysis PPACK-liposomes were mixed with **Cleanascite™** and subsequent centrifugation occurred resulting in PPACK separated from liposomes. This PPACK separated from liposomes was quantified using reverse-phase high-performance liquid chromatography (RP-HPLC) quantification of uncoupled PPACK. In vitro models of PPACK-liposomes prolonged arterial occlusion time and demonstrated an inhibitory effect on thrombin in acute arterial thrombosis. Liposome's surface was coated with PPACK resulting in reduced clearance in vivo and localized anti-thrombin activity was sustaining without requiring systemic anticoagulation.

McIntyre, John A., et al. "Antiphospholipid autoantibodies as blood biomarkers for detection of early stage Alzheimer's disease." Autoimmunity0 (2015): 1-8.

R-RAA aPL is a biomarker for Alzheimer's disease (AD). R-RAA-aPLs are detected upon oxidizing agents being exposed to plasma, serum, cerbrospinal fluid (CSF) or immunoglobulin fractions. Research has shown R-RAA antiphospholipid antibody (APLs) are less in CSF and serum of AD than healthy controls. Authors McIntyre et al cite R-RAA-aPLs's in biomarker discovery research on mild cognitive impairment (MCI), AD and healthy controls. The experiment detected R-RAA aPL by ELISA. The R-RAA aPL in sera from AD diagnostic group were less than healthy controls, whereas the MCI group had increased R-RAA aPL activity. Larger samples may require proper sample preparation and such research is important to detect biomarkers on dementia of Alzheimer's type. Authors cite **Cleanascite™**, "Aliquots of the 90 ADNI serum samples were thawed and treated with Cleanascite™ (Biotech Support Group, Inc.) at a serum: **Cleanascite™** ratio of 4:1 v/v in 2 ml microcentrifuge tubes with gentle rocking at 37 °C for 10 min".

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

Scientists analyzed impact of passively administered IgG subclasses to mice deficient in Th1 cytokine interleukin-12 (IL-12), the proinflammatory cytokine IL-6, or the Th2 cytokines IL-4 and IL-10 against cryptococcal infection. In the study variable-region-identical IgG1, IgG2a, IgG2b, and IgG3 monoclonal antibodies were analyzed against intravenous infection with*C. neoformans* in mice genetically deficient in interleukin-12 (IL-12), IL-6, IL-4, or IL-10. For the purification of monoclonal antibodies, IgG3 hybridoma and IgG1, IgG2b, IgG2a switch variants of MAb 3E5 developed from in vitro isotype switching were injected with ascites fluid from hybridoma cells. Cleanascite™ was successfully used for removing lipids and cell debris and the ascites fluid was sterilized. The antibody concentration was measured by enzyme-linked immunosorbent assay (ELISA).

Pan, Dipanjan, et al. "Anti-Angiogenesis Therapy in the Vx2 Rabbit Cancer Model with a Lipase-cleavable Sn 2 Taxane Phospholipid Prodrug using αvβ3-Targeted Theranostic Nanoparticles." *Theranostics* 2014; 4(6):565-578

Research involved developing and characterizing a Sn 2 lipase-labile prodrug of docetaxel Dxtl-PD. Excessive serum and lipase surrounding taxane prodrug in PFC nanoparticles was optimized using **Cleanascite™**, a lipid

removal reagent. In vitro experiment on anti-angiogenic molecule docetaxel prodrug in perfluorocarbon (PFC) nanoparticles cites Cleanascite™ for lipid removal from plasma-enzyme-nanoparticle mixture. Excessive lipids in samples interferes in high performance liquid chromatography. Subsequent to sample pretreatment by implementing the **Cleanascite™** protocol, the mixture is centrifuged and supernatant is analyzed by HPLC.

Bile

Megger, Dominik A., et al. "One Sample, One Shot-Evaluation of sample preparation protocols for the mass spectrometric proteome analysis of human bile fluid without extensive fractionation." *Journal of Proteomics* (2016). http://dx.doi.org/10.1016/j.jprot.2016.11.021

In brief, the article's authors report methods to overcome the biological variability of analyzing a high number of bile samples. They advance that easy sample preparation protocols are demanded representing a compromise between proteome coverage and simplicity in this study. For this, they evaluated the performance of simple workflows allowing for "one sample, one shot" experiments to identify biomarker candidates for various diseases of the hepatobiliary system. In detail, sixteen different protocols with modifications at the stages of desalting, delipidation, deglycosylation and tryptic digestion were examined. The article states "For delipidation, the **Cleanascite**™ Lipid Removal Reagent and Clarification Kit (BSG, NJ 08852, USA) was used following manufacturer's instructions.". The authors concluded that delipidation yielded a considerable number of complementary protein identifications and that **Cleanascite**™ treatment was indispensable for in-solution digestion methods.

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

Cholangiocarcinoma (CCA) is the malignant spread of biliary tree epithelial cells. Authors Danese et al describe the role of mucin5AC on bile samples of cancerous and non-cancerous patients. Biliary tract tumors, cholangiocarcinoma tissues, bile and serum samples express mucin 5AC (MUC5AC) glycoprotein. An enzyme-linked immunosorbent assay was performed to obtain MUC5AC quantification from bile and serum samples of extrahepatic cholangiocarcinoma and benign biliary diseases. MUC5AC expression as a serum/bile ratio was used to differentiate cholangiocarcinoma from cholangitis, cholangiocarcinoma from biliary stones and cholangitis from biliary stones. The article quotes "Delipidation was performed as follows: After centrifugation, the supernatant of each sample was mixed with 250 μL of **Cleanascite** (v/v ratio **Cleanascite** per sample = 1:4) and kept under mild agitation at 4°C for 1 hour to increase the agglomeration of fine lipids."

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

Authors RA Hauser-Davis and team cites **Cleanascite™** as an ideal lipid clarification reagent during sample preparation of fish bile containing matrix metalloproteinases (MMPs). Researchers identified matrix metalloproteinases in the bile of mullets (Mugil liza) and tilapias (Tilapia rendalli) which required clarification and purification studies prior to performing gel electrophoresis and zymography analysis. Lipid removal was performed using the delipidizer **Cleanascite™**, which is a non-ionic adsorbent, used to precipitate lipid fat droplets, cell debris and mucinous impurities.

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).

Cancer biomarkers allow differentiating malignant from nonmalignant biliary stenoses from bile samples via comparative proteomic analysis of bile. Bile samples were centrifuged and the supernatant was delipided with **Cleanascite™** followed by ultrafiltration. Comparative proteomic biomarker discovery experiments from bile samples of malignant or benign biliary stenosis identified 66 proteins and 7 proteins were elevated in malignant/nonmalignant disease. A cell surface protein, carcinoembryonic cell adhesion molecule 6 (CEAM6), which is associated with cancer was identified via immunoblot. ELISA confirmed CEAM6 as a clinically relevant cancer biomarker of biliary cancers.

Wang W, Ai KX, Yuan Z, Huang XY, Zhang HZ.Different Expression of S100A8 in Malignant and Benign Gallbladder Digestive diseases and sciences. 2012; DOI: 10.1007/s10620-012-2307-0 [epub ahead of print]

Cancerous and benign analysis of human bile requires analysis of biliary protein content to find biomarkers for early diagnosis of neoplasms, pancreatic cancer, cholangiocarcinoma. Bile contains high amounts of substances which interfere with protein separation and comparative analysis of bile samples. This study establishes **Cleanascite™** as a unique delipidation and sample preparation reagent. Proteolytic peptides from bile samples of patients with chronic calculous cholecystitis, gall bladder cancer, gall bladder adenomas were separated by two-dimensional liquid chromatography and identified by tandem mass spectrometry. The study results from the published article identified 544, 221, and 495 unique proteins from gallbladder adenoma, chronic calculous cholecystitis, gallbladder cancer bile samples. S100A8 was identified as being overexpressed in gallbladder cancer bile samples as compared with benign gallbladder tissue. Unique proteins were identified and S100A8 was elevated in malignant gall bladder bile and cancerous tissues of tumor infiltrated immune cells. In this study authors Wang et al concluded, "Compared with benign gallbladder diseases, consistently elevated S100A8 levels in malignant gallbladder bile and tissue indicate that gallbladder cancer is an inflammation-associated cancer. S100A8 may be a biomarker for gallbladder cancer."

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

Using **Cleanascite™** scientists isolated and identified hydrophobic polypeptides in human bile and subsequently performed specialized reversed-phase chromatography and gel-filtration, and MALDI-TOF mass spectrometry, to identify a small subset of five proteins. Bile fluid was obtained by endoscopic retrograde cholangiopancreatography (ERCP) from a patient with cholangiocarcinoma. Unfractionated bile fluid was centrifuged and partially cleared supernatant was then mixed with 250 µl of **Cleanascite™** followed by rotation, centrifuged, clear away the formed lipid-micelles.

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

A reliable method for general clean-up of bile fluid samples, which is suitable for 2-DE, by which we built up 2-D. Bile fluid samples were obtained during surgical drainage procedures. For sample delipidation and purification, **Cleanascite™** from Biotech Support Group was used to remove debris, nucleic acid and mucins followed by rotation for 1 hour. Salts, lipids, nucleic acids and other contaminants, are bound in bile fluid, dramatically affects both reproducibility and resolution of 2-DE. The objectives of our study were to establish a reliable sample preparation method and 2-DE options suitable for comparative proteomic analysis of bile fluid.

Guerrier L, Claverol S, Finzi L et al Contribution of solid-phase hexapeptide ligand libraries to the repertoire of human bile proteins.Journal of Chromatography A.2007;1176(1-2):192-205

Scientists used Immobilized peptide ligand libraries to concentrate dilute bile. For the detection of low abundance proteins from bile required **Cleanascite™** for clarification.

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

Surgical drainage of bile fluid samples from patients with cholangiocarcinoma and cholelithiasis was collected, sonicated and centrifuged to remove debris, nucleic acid and mucins as a preliminary separation. **Cleanascite™** was used for sample delipidation allowing for proper sample preparation process suitable for two-dimensional electrophoresis of bile fluid. By doing so, bile fluid analysis and identification of biomarkers by 2-D biliary maps are visualized.

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

Researchers discovered large amounts of lipids, bile salts in bile fluid obtained by ERCP from patients with cholangiocarcinoma. To identify proteins in bile fractions researchers used **Cleanascite™** to precipitate lipids

from "unfractionated bile" followed by one-dimensional gel electrophoresis, lectin affinity chromatography and liquid chromatography tandem mass spectrometry.

Tissue Lysates

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

Scientist isolated M.tb genomic DNA from cultures grown in 7H9 broth, OADC, 0.1% Tween 80. After centrifugation, the bacterial pellet was resuspended in water, packed volume sterile glass beads and 5 mL phenol: CHCl3 (pH 8). After vortexing and mixing the samples, the aqueous phase was removed following centrifugation **Cleanascite™** from Biotech Support Group was used for removing lipids from samples. CHCl3:isoamyl alcohol (24:1), 500 µL **ProCipitate™** (Biotech Support Group), and 3M sodium acetate was added to sample for DNA precipitation.

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.

For the localized control of acute thrombosis, researchers have developed a new platform that uses PPACK (Phe[D]-Pro-Arg-Chloromethylketone) with nanoparticles that serves as thrombin-inhibiting surfaces at sites of acutely forming thrombi to prevent the effects of local clot inhibition. In this article, PPACK and PPACK nanoparticle inhibition of thrombin were assessed in vitro via thrombin activity against a chromogenic substrate. Also in vivo acute arterial thrombosis model demonstrated that PPACK nanoparticles outperformed both heparin and uncomplexed PPACK in inhibiting thrombosis. The article mentions how **Cleanascite™** is used: "The extent of PPACK coupling was determined by reverse-phase HPLC quantification from uncoupled PPACK after centrifugation of nanoparticles with **Cleanascite™** lipid adsorption reagent from Biotech Support Group."

R. Kenneth Czambel, Alexander Kharlamov, Stephen C. Jones, Variations of brain endothelial nitric oxide synthase concentration in rat and mouse cortex, Nitric Oxide, Volume 22, Issue 1, 1 January 2010, Pages 51-57

To assess possible assay interference by lipids present in the homogenate matrix, samples were treated with **Cleanascite**™ (Biotech Support Group, North Brunswick, NJ), a commercially available lipid removal and clarification reagent. This reagent is a saline suspension of a solid-phase non-ionic adsorbent (pH 8.0) that selectively removes lipids from biological samples. Immediately prior to use, the **Cleanascite™** reagent was completely resuspended by gentle shaking. **Cleanascite™** (200 µL) was added to rat brain tissue homogenate (800 µL) and mixed for 20 min at room temperature by gentle shaking. Following centrifugation (1000g) for 20 min at 4 °C, the supernatant was carefully decanted into a clean collection vial and analyzed.

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

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.

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

Scientists measured β2GPI antigen (β2GPI: Ag), β2GPI aPA cofactor activity (β2GPI: Cof) and antibodies to β2GPI (αβ2GPI) from systemic lupus erythematosus (SLE) patients with aPAs (SLE-aPA +) and primary antiphospholipid syndrome (PaPS). Researchers implemented the **Cleanascite** protocol for studying β2 Glycoprotein-I (β2GPI), a cofactor for binding antiphospholipid antibodies and with *in vitro* anticoagulant properties in plasma.

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

As a lipid removal reagent, **Cleanascite™** was used to purify seven separate pancreatic cancer tissue samples which contained high lipid content. The researchers found that the sample delipidation provided by Cleanascite™ was necessary for studying the tissue homogenates. **Cleanascite™** was able to substantially improve the array quality of the pancreatic cancer tissue samples.

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

In vitro culture of human RBC spontaneously released protein factors that enhance T-cell growth and survival of normal and malignant activated T cells. RBC-CM generated from cultures of RBC reproduces the effectiveness of intact RBC in modulating proliferation, cell growth and survival of activated T cells. Often Sudan black staining does not detect lipids in the RBC-sup. Excessive lipids affect LC-MS results. For eliminating the possibility of lipid molecules which might be responsible for the bioactivity of red blood cells factors, researchers used **Cleanascite™** in the protocol. Following ultrafiltration and concentration of the in vitro assay of RBC-sup was quantified. For thermostability studies, the RBC-sup was boiled, concentrated and centrifuged preparing it for in vitro bioactivity assays.

Saliva/Tracheal swab/Sputum

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

Magnetic nanobeads amplification method based quartz crystal microbalance (QCM) immunosensor was tested for AI H5N1 virus detection. Captured H5N1 viruses by immobilized antibodies are measured by changes in frequency. Researchers used **Cleanascite**™ for selectively removing lipids, cell debris, lipoproteins, floating fats, impurities for pretreatment of samples prior to purification from tracheal swab samples for efficiently detecting pathogenic avian influenza (AI) H5N1 virus.

Nucleic Acid Isolation

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. **DOI:** 10.1186/s13104-016-2084-5

In brief, the article's authors report a method for the reproducible and reliable isolation of miRNA from the albumen and yolk of chicken eggs. These methods will allow the investigation of epigenetic programming in chick development previously unknown, and how this impacts the nutritional value of eggs for human consumption. The article states "…400 µl aliquots of the yolk/lysis solution was dispensed into five 1.5 ml microcentrifuge tubes. To each of these aliquots 600 µl of **Cleanascite**™ was added followed by rigorous vortexing until the sample became homogenous. The **Cleanascite**™ removes the lipid from this high fat tissue that would otherwise interfere with the extraction process…Solutions were then incubated at 4 °C for 1 h.".

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

Researchers from the Pacific Tuberculosis and Cancer Research Organization and Centers for Disease Control and Prevention used **Cleanascite™** during sample preparation for sequencing the complete genome of Mycobacterium tuberculosis H37Rv.

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

Cleanascite™ was used for RNA isolation from M.tuberculosis(MTB) samples. MTB mRNA are frequently analyzed for chemotherapy efficacy. Measuring acid fast bacilli positive strains and positive sputum culture conversion to negative before and after chemotherapy, allows scientists to measure the bactericidal effect and develop innovations in design of clinical trials for new treatments. Producing MTB RNA from small volume of sputum requires isolating RNA. **Cleanascite™** was added to the aqueous phase of the sample, followed by centrifugation and supernatant removal.

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

Authors of this article isolated mycobacterial RNA from specimens developing molecular markers for quantification of mRNA as a response for monitoring response to chemotherapy and for assessing the efficacy of new drugs for suspected multidrug-resistant tuberculosis. Levels of a stable and abundant structural RNA, 16S rRNA are isolated and added to homogenized sputum into a matrix tube for cell. After spinning and processing, 200 µl of chloroform was added and the aqueous and organic layers were separated by microcentrifugation. The aqueous phase which has the RNA was removed and 100 µl **Cleanascite™** is used to purify the sample. The aqueous phase is extracted with 500 µl chloroform:isoamyl alcohol and RNA is precipitated extracted with phenol and CHCl3, precipitated with isopropanol, and resuspended in a final volume.

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

Cell Culture Lysates

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

In brief, 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".

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

For hemoglobin assays it is necessary to remove lipidsResearchers are increasingly interested in the production of recombinant human hemoglobin from Escherichia coli and yeast hemoglobin which maintains current good manufacturing practices. Sample preparation provides effective methods for enriching and depleting hemoglobin from complex samples. Subsequent to sample preparation the samples produced maintain hemoglobin stability, mitigate iron-catalyzed reactions, and hemin production as well as expression and purification protocols are of high quality. Authors Liu et al published an article in the journal Metabolic Engineering and the article cites lipid removal agent **Cleanascite™** for sample clarification of hemoglobin assays. The article describes experiments on SDS-PAGE with heme and globin expression, globin patterns, heme, corproporphyrin and porphyrin levels, batch fermentations with heme and hemoglobin production levels The article quotes "Lipid removal agent **Cleanascite** (X2555-100, BIOTECH SUPPORT GROUP) was added according to product description in case of unclear suspensions in the supernatant. Protein concentration was measured by BCA protein assay kit".

Pan, Dipanjan, et al. "A strategy for combating melanoma with oncogenic c-Myc inhibitors and targeted nanotherapy." *Nanomedicine* 10.2 (2015): 241-251.

Research article in the journal NanoMedicine cites Biotech Support Group's **Cleanascite™** sample preparation reagent to obtain lipid clarified c-Myc inhibitor prodrug used in a drug delivery and enzymatic release platform for melanoma antiproliferation. Samples composed of human and mouse cell lines containing Sn-2 lipase-labile Myc inhibitor prodrug on a αvβ3-targeted nanoparticle platform to deliver c-Myc inhibitors were researched as an alternative to small-molecule inhibitors of c-Myc–Max. Lipid clarification was performed on synthesized Sn-2

lipase-labile Myc inhibitor prodrug in two αvβ3-targeted nanoparticle platforms (20 and 200 nm). Subsequent nanodelivery of c-Myc inhibitors to prevent melanoma progression requires sample preparation. Melanoma antiproliferation potency of lipid containing and lipid clarified human and mouse melanoma cell lines was obtained using Biotech Support Group's **Cleanascite™**.

Patents

US Patent 20130011413: Method and Pharmaceutical Composition for Treatment of Intestinal Disease

Inventors Iwakura Yoichiro, Kakuta Shigeru and Suzuki, Shunsuke and assignee The University of Tokyo published United States Patent Application 20130011413 titled, Method and Pharmaceutical Composition for Treatment of Intestinal Disease. The patient details a method for using interleukin-related substances like an IL-17F inhibitor typified by an anti-IL-17F antibody to treat intestinal disease such as colon polyps or colorectal cancer. The patent cites **Cleanascite™** from Biotech Support Group in a method for the selection of neutralizing antibodies to mouse IL-17F and IL-17A, and to remove lipid and lipid like material from the recovered culture supernatant.

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

Phospholipids such as phosphatidylcholines interfere with analyte ionization in electrospray MS detection by reducing analyte sensitivity referred to as ion suppression or matrix effects. The matrix effect is the ion-suppression and/or enhancement observed in electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) during LC-MS use. Such interference is reduced with **Cleanascite** sample preparation reagent. Pretreatment of samples reduces interferences of contaminants and increases accuracy of analytes during bioanalytical testing and quantitation methods such as liquid chromatography-mass spectrometry/mass spectrometry. For example, matrix effects or ion suppression caused by phospholipids (phosphatidylcholines) in mass spectrometry results in low recovery and high variation of results. Thus **Cleanascite™** provides a rapid procedure for the removal of phospholipids and interfering analytes causing matrix effects and proteins from a bioanalytical sample prior to performance of analytical procedures.

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

Researchers used **Cleanascite™** for delipidation of sera followed by proteinase K treatment to determine cancer specific bands from samples used for developing monoclonal antibodies which specifically recognize the tumor cell specific NADH:protein thiol reductase and hybridoma cell lines. SDS Page and Western blot analysis of sera from cancer patients (with prostate, lymphoma, ovarian, leukemia, breast) and health patients followed.

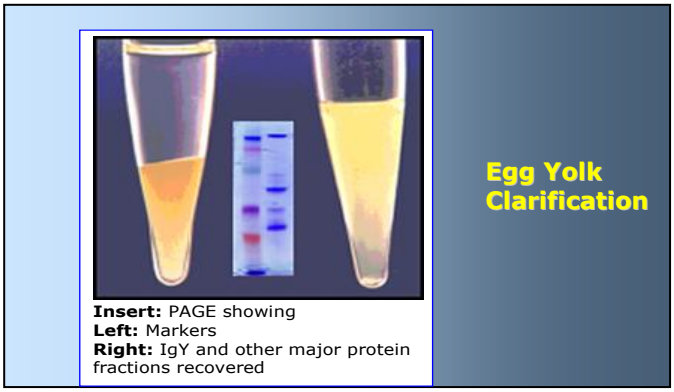
Mcintyre, John A. United States Patent: 20120107841. Serum Diagnostic Method, Biomarker and Kit for Early Detection and Staging of Alzheimer's Disease

Investigators are researching redox-reactive autoantibodies (R-RAA) present in serum which unmask antigen recognition sites upon oxidative exposure. Using ELISA to quantitatively measure concentration of unmasked antibodies in serum samples in vitro by the presence of their recognition epitopes, scientists could assess the increase in R-RAA over their base line values and compare it to the increased ELISA reactivity in AD and/or normal individuals. Comparisons between the AD and normal populations revealed highly significant differences in R-RAA antiphosphatidylethanolamine (aPE). Essentially the autoantibodies are detected in blood with an oxidizing agent and then using a screening assay to detect antibodies that bind a self antigen. Hemin is the oxidizing agent used for R-RAA aPE ELISA. The inventors developed technology to 'unmask' these autoantibodies in serum samples in vitro and matched AD specific epitopes to reactive autoantibodies. The increase in R-RAA aPE in MCI serum samples and changes in hippocampal choline acetyltransferase (ChAT) in end-stage AD or MCI are being exploited as potential biomarkers.This patent cites **Cleanascite™** for lipid removal and clarification: "For the preparation of serum samples, **Cleanascite™** from Biotech Support Group was added. Specifically 1:4 vol/vol of **Cleanascite™** is added to each serum sample in a 2 ml micro tube, using a pipette tip".

Cleanascite™

Lipid Adsorption & Clarification

- Effectively replaces chlorinated/fluorinated hydrocarbons (eg. freon)
- Workflows for antibodies, proteins, nucleic acids, proteoglycans, and most serum analytes
- Ideal for clarifying ascites, serum, cell & tissue culture, and organ homogenates
- Clarifies bile and saliva
- Extensively cited in journal articles
- Extends the life of membrane and chromatographic apparatus.



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

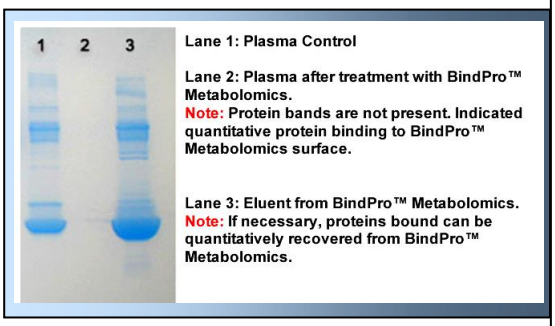
Product	Quantity (ml)	Process Volume (ml)*	Item No.
Cleanascite™	10	40	X2555-10
Cleanascite™	50	200	X2555-50
Cleanascite™	100	400	X2555-100
Cleanascite™	1000	4000	X2555-1000

*Based on typical volume ratios, can be adjusted to lipid load

BindPro™ & BindPro™ Metabolomics

Aqueous Protein Removal & Enrichment of Metabolites & Analytes

- Serum and plasma protein removal, >95%
- Aqueous protein crash
- < 30 minute protocol
- Applicable for drug binding/screening and metabolomics



Lane 1: Plasma Control

Lane 2: Plasma after treatment with BindPro™ Metabolomics.
Note: Protein bands are not present. Indicated quantitative protein binding to BindPro™ Metabolomics surface.

Lane 3: Eluent from BindPro™ Metabolomics.
Note: If necessary, proteins bound can be quantitatively recovered from BindPro™ Metabolomics.

Product	Qty	Item No.
BindPRO™ Metabolomics	15 Preps*	BPM55-15
BindPRO™ Metabolomics	50 Preps*	BPM55-50
BindPRO™	15 ml	BP355-15

*Based on 20-30 µl serum

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