BIOTECH SUPPORT GROUP

Cleanascite™

Lipid adsorption and clarification reagent

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

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

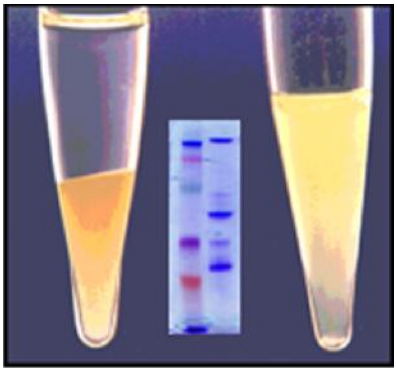
Removes Lipid Factors
Phospho-Lipids
>99% Cholesterol &
Triglycerides
Lipoproteins
Extracellular Vesicles
(Exosomes)

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

Improved Assay Performance

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

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



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

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

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

Protocol

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

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

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

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

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

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Selection of Reference Applications

Cell Response

Slattery, Karen, et al. "Uptake of lipids from ascites drives NK cell metabolic dysfunction in ovarian cancer." *Science Immunology* 10.107 (2025): eadr4795. The investigators depleted ascites of lipids using a lipid adsorption and clarification reagent which caused widespread lipid depletion in ascites"; "Lipids were depleted from FCS and ascites samples using **Cleanascite lipid removal reagent**"

Flury, Anna, et al. "A neurodegenerative cellular stress response linked to dark microglia and toxic lipid secretion." *Neuron* (2024).

The brain's primary immune cells, microglia, are a leading causal cell type in Alzheimer's disease (AD). Yet, the mechanisms by which microglia can drive neurodegeneration remain unresolved. The integrated stress response (ISR), characterizes a microglia subset with neurodegenerative outcomes. Mechanistically, evidence is presented that ISR activation promotes the secretion of toxic lipids by microglia, impairing neuron homeostasis and survival *in vitro*. **Notably, eliminating lipids using an adsorbent resin (Cleanascite) abolished these toxic effects.**

Wang, H., Yung, M.M., Xuan, Y. *et al.* Polyunsaturated fatty acids promote M2-like TAM deposition via dampening RhoA-YAP1 signaling in the ovarian cancer microenvironment. *Exp Hematol Oncol* **13**, 90 (2024). https://doi.org/10.1186/s40164-024-00558-8. Tumor-associated-macrophages (TAMs) are multi-polarized. However, the impact of omental conditioned medium/ascites (OCM/AS) on TAM polarization and its function in tumor progression remains elusive. The article states "Recent studies have suggested that lipid accumulation and metabolism are associated with the differentiation and activation of protumoral TAMs. Hence, we examined the lipid droplets (LDs) in MΦs, which are cellular storage organelles for neutral lipids. …**the removal of free fatty acids in OCM by Cleanascite™ attenuated LDs deposition in OCM-C MΦs**, indicating M2 MΦs and OCM-MΦs exhibited higher lipid accumulation and metabolism….Likewise, we demonstrated that the cellular ROS levels in OCM/AS-MΦs derived from PBMC MΦs were significantly upregulated, **whereas the addition of Cleanascite™ mitigated the increased ascites-mediated ROS level**, indicating that the accumulation of intercellular UFAs is responsible for the enhanced ROS production in OCM-MΦs." The article goes on to state "**In contrast, removing lipids by Cleanascite™ remarkably prevented the reduction of YAP1 in OCM-MΦs**.

Zhang, T., Zhao, F., Hu, Y. *et al.* Environmental monobutyl phthalate exposure promotes liver cancer via reprogrammed cholesterol metabolism and activation of the IRE1α-XBP1s pathway. *Oncogene* (2024). https://doi.org/10.1038/s41388-024-03086-1

Abnormalities in lipid metabolism is a molecular hallmark for not only cancer cells, but also tumor-associated immune cells. To study those abnormalities, the article states " To investigate the role of lipids in regulating the macrophage function, **we first employed Cleanascite to remove all lipids and lipoproteins from the conditioned media (CM)** of HepG2 cells. Interestingly, lipid-depleted HepG2 CM significantly suppressed the migration ability of THP-1PMA cells both in control and MBP-exposed groups. Also, the expression of M2-type markers (such as CD206, CD163, and ARG1) in THP-1PMA cells dramatically decreased after lipid clearance, while the expression of M1-type markers was up-regulated…"

Albakri, Marwah M., et al. "Fatty acids secreted from head and neck cancer induce M2-like Macrophages." Journal of Leukocyte Biology (2022). To assess depletion of fatty acids from tumor supernatants, tumor-conditioned medium was treated with Cleanascite according to the manufacturer's instructions and prior to incubation with monocytes. The article states "**Depletion of Fatty acids with Cleanascite from FaDu or SCC supernatants largely reversed the phenotypic changes in Macrophage**s otherwise observed by incubating monocytes in these supernatants".

Chan, David, et al. "Polyunsaturated Fatty Acids Promote Protumoral Macrophage Polarization via a RhoA-YAP1 Signaling Pathway in the Ovarian Cancer Microenvironment." (2022).
The article states "To selectively remove lipids, Cleanascite™ Lipid Removal Reagent (Biotech Support Group) was added to the omental conditioned medium (OCM) according to the manufacturer's suggestions. **... Intriguingly, the removal of free fatty acids in OCM by the Cleanascite™ attenuated lipid droplets deposition in M2-like MΦs and OCM-MΦs** (Fig. 4A), indicating protumoral M2-like TAMs exhibited higher lipid accumulation and metabolism in the fatty acid-enriched OCM or the malignant ascites."

Monoclonal Antibodies/Ascites
Testa, Anna, et al. "TARGETING THE αVβ3/NgR2 PATHWAY IN NEUROENDOCRINE PROSTATE CANCER." *Matrix Biology* (2023). **The article states "LM609 purification was performed as previously described. Briefly, LM609 ascites was cleaned with Cleanascite** …, and recirculated over a Protein A column."

Biofluids
Arora, Pearl, et al. "Nanopore-based detection of periodontitis biomarker miR31 in saliva samples." *Electrophoresis* (2024).

Aberrations in the microRNAs (miRNA) levels have been the cause behind various diseases, including periodontitis. In this study, the researchers developed a label-free, real-time sensing method for the detection of miR31. When human saliva was tested however, extended pore blockage with large noise levels was noted due to the presence of interfering lipid or lipoprotein complexes. To circumvent this, the article states "we found that that the nonspecific pore blockage by the sample matrix could be significantly diminished **by simply treating human saliva samples with Cleanascite. ...By taking advantage of Cleanascite treatment**, the saliva samples produced current modulations with similar signatures to that of the miR31-p5 duplex in the control (i.e., in water solutions)."

Qin WH, Liu JT, Wang SP, Yang ZS, Wang KK, Hu B. Antibody array-based proteomic screening of novel biomarkers in malignant biliary stricture. Cancer Biomark. 2022;33(3):359-368. doi: 10.3233/CBM-210095. PMID: 34542063.
The article states "…bile was collected upstream from the biliary stricture, then mixed with PBS containing 0.1% Tween 20 and a protease inhibitor cocktail **..., the supernatant was mixed with Cleanascite™ (Biotech Support Group, NJ, USA) to remove lipids.** The article concludes that twenty proteins were found differentially expressed in malignant versus benign biliary strictures…"

Plasma/Serum
Huneault HE, Lo JS, Bai S, He Z, McPhaul MJ, Bril F, et al. Fasting intact insulin by mass spectrometry is associated with metabolic dysfunction-associated steatotic liver disease in youth . Hepatol Commun. 2024;8: e0582. https://doi.org/10.1097/ HC9.0000000000000582. This study sought to determine whether fasting intact insulin, measured by liquid chromatography-tandem mass spectrometry, is associated with MASLD in children. The methods describe "Quest Diagnostics Nichols Institute performed measurements of intact insulin and C-peptide… the samples were plated and mixed with the addition of internal standards (bovine insulin and a stable isotopically labeled [13C/15N] C-peptide) **and Cleanascite delipidation reagent**."

Zheng, Wenshu, et al. " Nanopore-based disease diagnosis using pathogen-derived tryptic peptides from serum ." Nano Today 45 (2022): 101515. The article states "Treatment of digested serum samples with two commercial lipid removal agents revealed variable effectiveness in reducing these artifacts, with one reagent (LRA; synthetic calcium silicate hydrate) revealing little to no effect to reduce the frequency, duration, and extent of non-specific pore blockages…**Serum digests treated with the second reagent (Cleanascite™) demonstrated the absence of these artifacts**".

Chen, Chao, et al. "Obesity-driven oleoylcarnitine accumulation in tumor microenvironment promotes breast cancer metastasis-like phenotype." *Acta Pharmaceutica Sinica B* (2025). The article states **"For the delipidation of mouse serum, Cleanascite** was employed following the manufacturer's protocol designed specifically for serum samples.

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

CONTACT US

We welcome your questions and comments regarding our products.

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