Sino Biological

ProPure™

Excellence Built on Ultra-Low Endotoxin

ProPure™ Endotoxin-Free Protein Handbook

- Preclinical Studies
- Animal Immunization
- Cell-Based Assays
- Endotoxin-Sensitive Bioassays

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ProPure™ Endotoxin-Free Protein Handbook

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Endotoxin Overview

What is Endotoxin?

Endotoxin is a unique component of the outer membrane of Gram-negative bacteria, primarily consisting of Lipopolysaccharide (LPS). Endotoxins are widely present in the cell walls of various Gram-negative bacteria, such as Escherichia coli, Salmonella, Brucella, Typhoid bacillus, Proteus, etc. They may also exist in some fungi, rickettsia, and mycoplasma. Endotoxins are ubiquitous in nature, distributed in water, soil, and air, and also found in humans and animals.

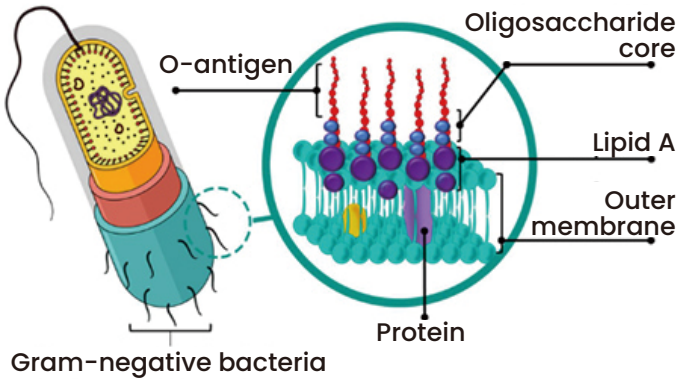


Figure 1. Endotoxin composition of Gram-negative bacteria.
(doi: 10.3390/toxins13080533)

Endotoxins reside in the outermost layer of the Gram-negative bacterial cell wall, which maintains cellular morphology and mediates host immune interactions. These bacteria possess an asymmetric double membrane: the inner leaflet contains phospholipids, while the outer leaflet consists of lipopolysaccharide (LPS). Although comprising only 10–15% of the outer membrane, LPS covers ~75% of its surface, forming an effective permeability barrier that enhances resistance to antimicrobial agents. Minimal endotoxin is released during normal growth, but substantial quantities are liberated upon cell lysis or death, causing fever, microcirculatory disturbances, endotoxic shock, and intravascular coagulation.

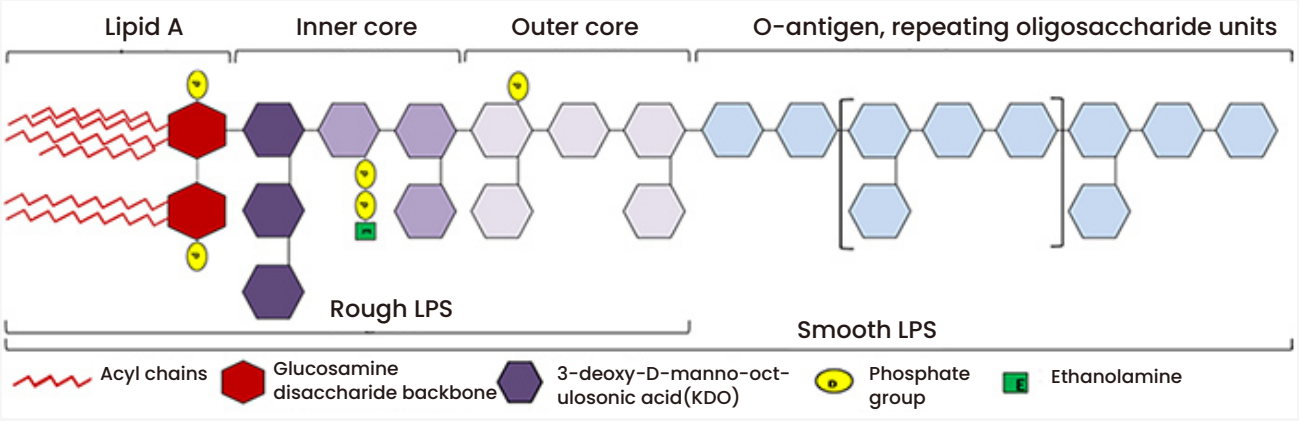


Figure 2. Structure of LPS. Lipid A: The hydrophobic anchor and primary toxic component that triggers immune responses; Core polysaccharide: A conserved oligosaccharide chain; O-antigen: The variable, outermost polysaccharide that determines bacterial serotype.
(doi: 10.1111/prd.12433)

The Impact of Endotoxin

Endotoxins possess immunostimulatory properties. By stimulating host cells to produce a large number of inflammatory cytokines, they trigger immune responses and may lead to inflammatory reactions. Therefore, during animal immunization experiments and cell culture processes, even trace amounts of endotoxin contamination can affect experimental outcomes. To ensure the safety of biological products and injectables, pharmacopoeias in various countries have established stringent endotoxin testing methods and permissible limit standards.

Endotoxin disrupts immunity and triggers inflammation

Endotoxins can trigger strong immune responses, including neuroinflammation via microglial activation, potentially affecting neurodegenerative diseases like Parkinson's. Sandiego C. and colleagues found that systemic LPS administration (1.0 ng/kg, i.v.) in healthy humans led to increased microglial activation, elevated peripheral inflammatory cytokines, changes in vital signs, and self-reported sickness symptoms, as shown by PET imaging.

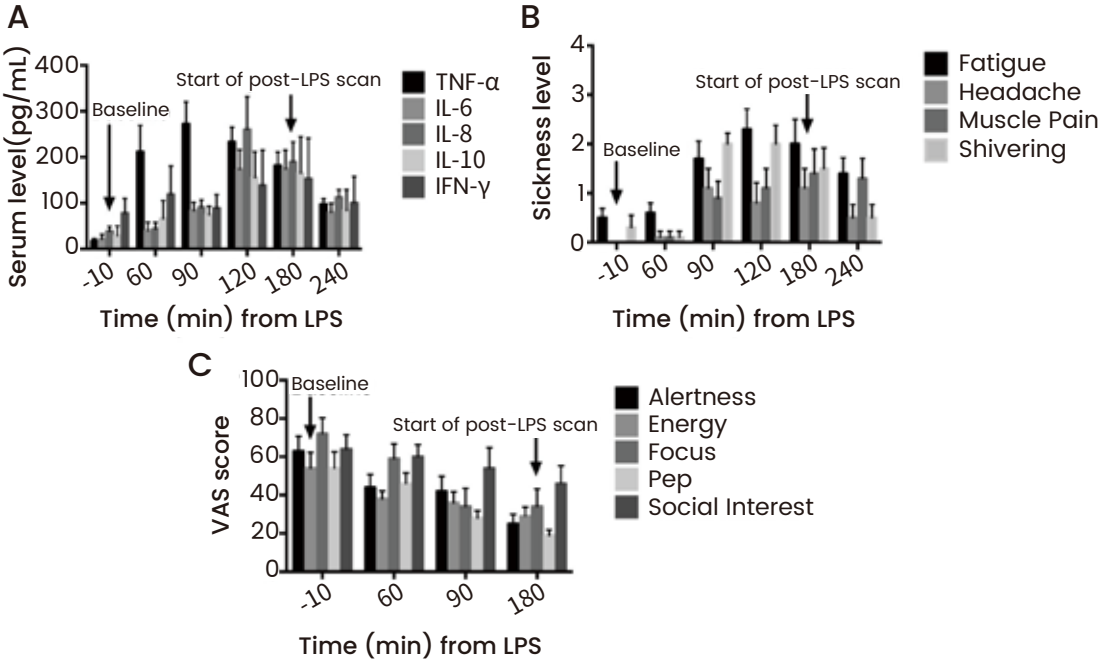


Figure 3. LPS administration significantly increased inflammatory cytokine serum levels and sickness symptom levels.
(doi: 10.1073/pnas.1511003112)

Endotoxin induces protein aggregation

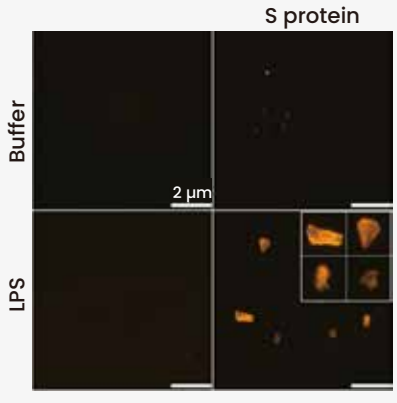


Figure 4. Jitka P. et al. demonstrated through fluorescence microscopy that LPS treatment markedly enhanced aggregation of S protein.These findings indicate that endotoxin can directly promote protein aggregation, potentially altering protein structure and function.
(doi: 10.1002/1873-3468.14490)

Endotoxin interferes with bioprocess development & Manufacturing

Endotoxins bind to natural proteins in plasma, saliva, and neutrophils, disrupting recombinant protein bioprocessing and complicating biopharma production. They affect protein binding affinity by: (1) directly binding to recombinant proteins, altering their conformation; (2) promoting protein aggregation, masking epitopes; and (3) competing for binding sites on target molecules. These issues impact immunology research, vaccine development, and therapeutic protein production, underscoring the need for stringent endotoxin control in biotech and biopharma workflows.

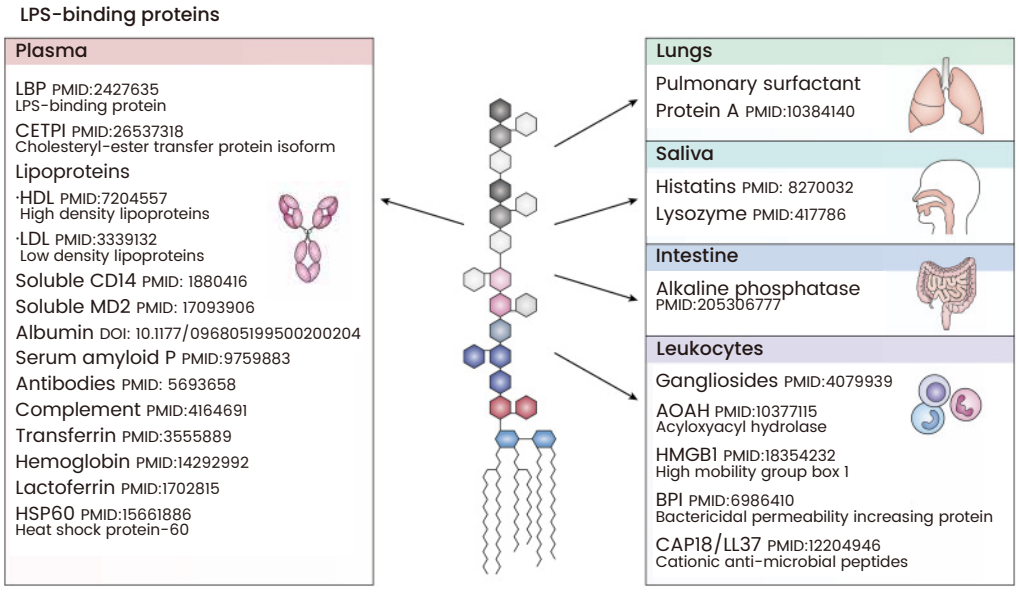


Figure 5. Proteins naturally binding to LPS are numerous
(doi: 10.1016/j.jbc.2023.105506)

Endotoxin disrupts sensitive cell cultures

Multiple cell types are susceptible to endotoxin, including macrophages, neutrophils, dendritic cells, lymphocytes, hepatocytes, platelets, and endothelial cells. Studies show that extremely low endotoxin concentrations disrupt normal cellular physiology. Schwarz H et al. found that LPS concentrations of 0.02–2 ng/mL activate immune responses, with varying cell sensitivities: THP-1 cells showed only IL-8 upregulation at 2 ng/mL, whereas moDCs secreted substantial IL-6, IL-8, IL-12, and TNF-α. Human monocytes and CD1c+ DCs were more sensitive, with 0.2 ng/mL LPS inducing all these cytokines.

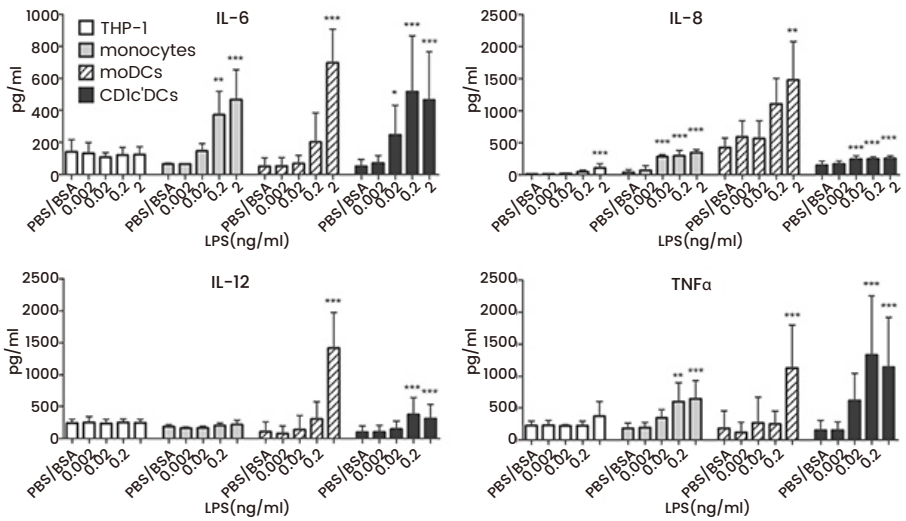


Figure 6. Effect of low-concentration LPS on cytokine production in different human immune cells.
(doi: 10.1371/journal.pone.0113840)

Ultra-low Endotoxin Proteins

Definition and Standards of Ultra-Low Endotoxin Proteins

Ultra-low endotoxin proteins are recombinant proteins manufactured and purified under stringent endotoxin-controlled processes, resulting in endotoxin levels significantly lower than those of conventional research-grade proteins. The goal is to minimize LPS interference in cell-based assays, animal models, and immunological studies. According to USP <85> bacterial endotoxins test, endotoxin levels must be below 0.5 EU/mL at batch release testing to be considered acceptable and safe.

Table 1. Detection Methods for Endotoxins

Method		Sensitivity (EU/mL)	Use Cases
Limulus Amebocyte Lysate (LAL)	Gel Clot	0.03-0.06	Routine screening, semi-quantitative
	Chromogenic	0.005	Complex matrices (e.g., serum, cell culture media)
	Turbidimetric	0.001	High-precision quantification (e.g., injectables)
Recombinant Factor C (rFC) Assay		0.01	Alternative to LAL, quantitative detection

Why Use Ultra-Low Endotoxin Proteins?

Endotoxin (LPS) is known to induce systemic and neuroinflammatory responses in animals, which can complicate experimental results and potentially invalidate preclinical data. Also, recombinant proteins contaminated with endotoxins may adversely affect cellular behavior due to their immunostimulatory and cytotoxic properties. Endotoxins are notorious contaminants in protein preparations. Even trace amounts of endotoxin in recombinant proteins can trigger potent immune responses, distort results, and compromise patient safety, especially in sensitive applications such as immunology, cell and gene therapy, and vaccine production. Ultra-low endotoxin proteins are therefore essential for ensuring experimental accuracy, safety, and regulatory compliance in sensitive biological and pharmaceutical applications.

To prevent adverse reactions caused by pyrogens, particularly bacterial endotoxins, health authorities such as the FDA set pyrogenic thresholds to ensure the safety of injectable drugs, medical devices, and other products that come into contact with the human body.

Table 2. Standard thresholds of endotoxin level for various biomedical applications.

Application	Endotoxin Limit	Notes
Cerebrospinal fluid contact devices	0.06 EU/mL or 2.15 EU/device	Whichever is less
Intrathecal drugs	0.2 EU/kg/hour	For drugs administered into cerebrospinal fluid
Single-use systems	0.25 EU/mL	Recommended for most systems, but not a blanket limit
Water for Injection (WFI)	0.25 EU/mL	Pharmacopeial limit
Cardiovascular/lymphatic system devices	0.5 EU/mL or 20 EU/device	Whichever is less
Intravenous (IV) or Intramuscular (IM) drugs	5 EU/kg/hour	Most common limit for parenteral drugs
Non-intrathecal radiopharmaceuticals	175 EU/V	V is the maximum recommended dose in mL
Most parenteral drugs	5 EU/kg/hour	For non-intrathecal administration
Medical devices (general)	20 EU/device	For devices labeled as non-pyrogenic
Medical devices (cerebrospinal fluid contact)	2.15 EU/device	For devices contacting cerebrospinal fluid
Intraocular ophthalmic devices	0.2 EU/device	Generally recommended, may be lower

Major Applications of Ultra-Low Endotoxin Proteins

Ultra-low endotoxin proteins are critical for endotoxin-sensitive applications, where even minimal contamination can compromise data accuracy, animal welfare, or human safety. Key applications include animal immunization, research on immune and inflammatory mechanisms, disease modeling, cell therapy manufacturing, preclinical drug development, cell-based assays, and therapeutic protein development.

Animal immunization/Antibody development

During animal immunization or in vivo experiments, the inflammatory response to endotoxins can lead to endotoxemia in test animals. This may halt further progress and invalidate the entire study. Endotoxins bind to surface receptors, such as TLR4 on macrophages and dendritic cells, triggering the production of pro-inflammatory cytokines that are not induced by the target protein. This can mask the true pharmacological effects and toxicity of the antibody drug, leading to biased experimental results that affect subsequent clinical dose design and safety assessments.

Cell therapy

Ultra-low endotoxin proteins are essential for T cell expansion and functional assessment, CAR construct development, and in vitro functional validation. Immune cells and stem cells are exquisitely sensitive to endotoxin—trace amounts can cause unintended activation or exhaustion, alter cell phenotypes and differentiation trajectories, and compromise the safety and efficacy of final products.

Subfield	Application	Notes
CAR-T Cell Therapy	Cytokines for T cell activation, expansion, and differentiation (e.g., IL-2, IL-7, IL-15, IL-21)	Endotoxin activates TLR4 on T cells, causing nonspecific activation and exhaustion, compromising clinical efficacy and safety. <0.25 EU/mL (culture medium); advanced standard <0.1 EU/mL
Stem Cell Therapy	Growth factors for culturing mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs)	Endotoxin induces MSCs to differentiate into inflammatory phenotypes, losing immunomodulatory function. <0.1 EU/mL

Preclinical drug development and in vivo studies

· **In vivo Pharmacokinetics/Pharmacodynamics (PK/PD) and Safety Assessment:** When recombinant proteins are administered as drug candidates in animal models, ultra-low endotoxin prevents pyrogenic reactions or systemic toxicity, ensuring observed effects derive solely from the protein's bioactivity.

· **Disease Modeling:** Prevents endotoxin-induced nonspecific inflammation from confounding experimental results.

· **Toxicology Studies:** Meets pyrogen limits set by regulatory agencies such as the FDA.

Immune and inflammatory mechanism research

Ultra-low endotoxin proteins offer advantages in macrophage/dendritic cell function studies, T/B cell activation and functional assessment, inflammatory cytokine detection, and inflammation-related signaling pathway research.

· **TLR4 Signaling Studies:** Prevents false positives from endotoxin (LPS) contamination.

· **Macrophage Polarization Research:** Ensures precise, reproducible dose-response curves using defined LPS concentrations.

· **Immune Checkpoint Studies:** Required for ultra-low endotoxin background in assays involving PD-1/PD-L1 antibodies and similar targets.

Neurodegenerative disease research

In neurodegenerative disease research, endotoxin alone can activate microglia, confounding studies of pathological mechanisms. Ultra-low endotoxin proteins are therefore essential for constructing Alzheimer's disease models, investigating protein aggregation and neuroinflammation, and analyzing microglial activation.

Vaccine development

· **mRNA Vaccines:** Plasmid DNA used as transcription templates must be endotoxin-free to ensure transfection efficiency and immunogenicity.
· **Recombinant Protein Vaccines:** Strict endotoxin control required for adjuvants/antigens.
· **Viral Vector Vaccines:** rAAV standards: typically <10 EU/mL; premium grade <2.5 EU/mL.

Therapeutic protein development

· **Monoclonal Antibody Production:** Culture media additives must be ultra-low endotoxin.
· **ADC Drugs (Antibody-Drug Conjugates):** Target proteins require ultra-low endotoxin to ensure safety.
· **Fusion Protein Drugs:** GMP-grade raw materials for final drug production.

Organoids and 3D cell culture

Ultra-low endotoxin proteins are critical for organoid culture to prevent endotoxin-induced cytotoxicity, inflammation, or aberrant differentiation.

· **Tumor Organoid Drug Screening:** As "patient avatars," tumor organoids require high-purity, bioactive proteins to ensure accurate reflection of patient tumor biology.
· **Organ Development Models:** Organoid cultures demand ultra-low endotoxin conditions.
· **Tissue Engineering:** Immobilize ultra-low endotoxin growth factors on scaffolds to prevent rejection and inflammation in bone, skin, and cartilage regeneration.

High-Sensitivity Detection and Functional Screening Platform

High-Throughput Cytokine Screening, Cell Proliferation/Differentiation Bioactivity Assays

Sino Biological ProPure™ Endotoxin-Free Proteins Advance Scientific Research

To address the rigorous requirements of researchers and product developers in in vivo modeling, cell therapy, and drug discovery, Sino Biological offers high-purity ProPure™ endotoxin-free proteins and customized production services. These endotoxin-free recombinant proteins are essential for advancing scientific research and drug development, enabling accurate and reliable results in endotoxin-sensitive applications.

Sino Biological's Center for Bioprocessing (C4B) in Texas produces ProPure™ endotoxin-free proteins with levels below the quantification limit (BQL) (< 0.05 EU/mg), which exceed the industry standard (0.5 EU/mg according to USP <85>), best for pre-clinical animal studies, accurate cell assays, and detection assays.

Sino Biological ProPure™ Endotoxin-Free Protein

Sino Biological proudly launches ProPure™ Endotoxin-Free Recombinant Proteins. Manufactured at our US Bioengineering Center (C4B) using advanced purification technologies, this series ensures minimal endotoxin levels to reduce immunogenic interference—providing reliable reagents for immunology research and biopharmaceutical development.

ProPure™

ProPure™ Endotoxin-Free Protein Advantage

Endotoxin levels < 0.05 EU/mg, significantly exceeding industry standards and markedly reducing non-specific immune responses triggered by endotoxins.

· Integrate endotoxin control throughout the entire production process.
· Strict test and control: Endotoxin testing using the rFC assay
· Ready to use, no reconstitution is needed.
Ideal for animal immunization, cell-based assays, and preclinical studies
* For all ProPure™ products, we guarantee endotoxin levels < 0.5 EU/mg.

Sino Biological's Endotoxin-Free Guarantee

- Endotoxin-free plasmids
- Endotoxin-free buffer with filtration
- Low endotoxin-affinity plastic containers
- Regular Clean-in-place (CIP)

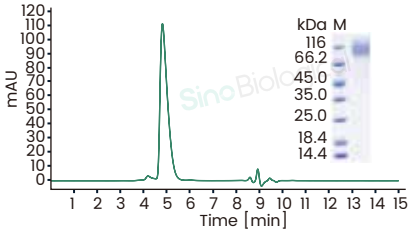
Applications of ProPure™ Endotoxin-Free Protein

- Preclinical Studies
- Animal Immunization
- Cell-Based Assays
- Any Sensitive Bioassays

Featured Products

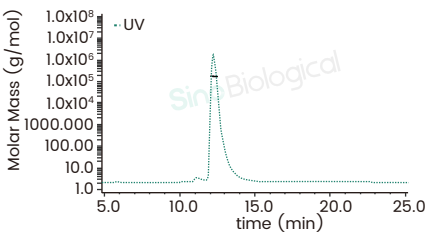
Human CEACAM-5/CD66e Protein (His Tag), Endotoxin < 0.05 EU/mg | Cat#: 11077-H08H-UE

High Purity



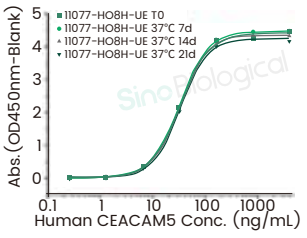
Purity ≥ 95% as determined by SDS-PAGE & SEC-HPLC.

MALS-validated



The purity of Human CEACAM-5/CD66e Protein (His Tag) was more than 95% and the molecular weight of this protein is around 184.5 kDa verified by SEC-MALS.

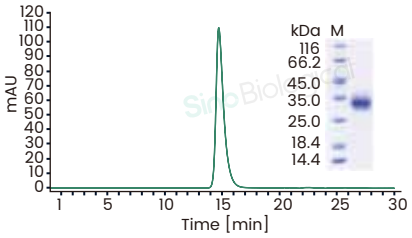
High Stability



Immobilized Anti-CEA Antibody (Research Grade Labetuzumab Biosimilar) at 2 μg/mL (100μL/well) can bind Human CEACAM-5/CD66e Protein (His Tag). The protein exhibited stable performance after 21 days of storage at 37°C, as measured on Day 0, Day 7, Day 14, and Day 21.

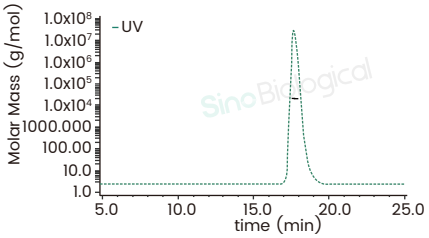
Human CD40/TNFRSF5 Protein (ECD, His Tag), Endotoxin < 0.05 EU/mg | Cat#: 10774-H08H-UE

High Purity



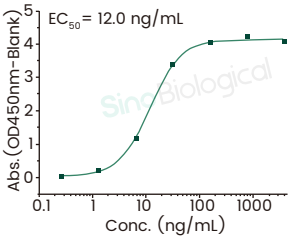
Purity ≥ 95% as determined by SDS-PAGE & SEC-HPLC.

MALS-validated



The purity of Human CD40/TNFRSF5 Protein (ECD, His Tag) was more than 95% and the molecular weight of this protein is around 26.9 kDa verified by SEC-MALS.

Activity verified by ELISA



Immobilized Human CD40/TNFRSF5 Protein (ECD, His Tag) at 2 μg/mL (100 μL/well) can bind Human CD40 Ligand (ECD, hFc & AVI Tag), Biotinylated (Cat#: 10239-H42H-B). The EC_{50} is 12.0 ng/mL.

More ProPure™ Products

Molecule	Cat#	Species	Purity	Endotoxin	Activity
EGFR	10001-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
PD-L1/B7-H1	10084-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
HER3/ERBB3	10201-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
PD-1	10377-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
CD16a/FCGR3A	10389-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
TROP-2	10428-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
PCSK9	10594-H08H1-UE	Human	SDS-PAGE/SEC-HPLC verified	< 0.5 EU/mg	Active
CD40/TNFRSF5	10774-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
CTLA-4	11159-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.05 EU/mg	Active
FOLR1	11241-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
c-Kit	11996-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.05 EU/mg	Active
Siglec-3/CD33	12238-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.05 EU/mg	Active
LAG-3	16498-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.05 EU/mg	Active
Nectin-4	19771-H08H-UE	Human	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.5 EU/mg	Active
FOLR1	90950-C08H-UE	Cynomolgus, Rhesus	SDS-PAGE/SEC-HPLC/SEC-MALS verified	< 0.05 EU/mg	Active

Explore More ProPure™ Endotoxin-Free Protein

Discover Sino Biological's ProPure™ Endotoxin-Free Proteins—engineered for superior quality to deliver more reliable research outcomes.

- Drug Targets
- ADC Targets
- Cell Therapy-Related
- Immune Checkpoints
- TNF Family
- Cytokines
- Cytokine Receptors
- Virus Antigens
- Fc Receptors / Ig-Related
- More...

Discover More ProPure™

ProPure™ Endotoxin-Free Protein/Antibody Production Service

Sino Biological's Center for Bioprocessing (C4B) provides custom endotoxin-free protein expression services tailored for stringent preclinical and drug development requirements. As a local production hub, C4B offers comprehensive and scalable manufacturing of endotoxin-free proteins and antibodies. It uses optimized mammalian expression systems, proprietary design, and multi-step purification. This delivers bioactive, high-purity products ideal for sensitive applications, such as animal immunization and preclinical research.

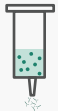
Integrate endotoxin control throughout the entire production process


Endotoxin-free plasmids


Endotoxin-free buffer with filtration


Low endotoxin-affinity plastic containers


Mammalian expression system


Advanced purification technology


Regular Clean-in-place (CIP)

Service Highlights

Customized antibodies & proteins delivered in as fast as 2 weeks

- Mammalian platforms: Mammalian expression system to ensure minimal endotoxin levels
- Scalable production: 24 well plates to 25 L bioreactors for your screening to production level protein needs
- Superior quality: >90% purity by SEC-HPLC, <0.05 EU/mg endotoxin level

Quote Now

Endotoxin-Free Protein Expression Service Details

Item	Details	
End-to-End Expression & Purification Comprehensive QC	• Multiple expression systems: HEK293, ExpiCHO, Expi293 optimized for low-endotoxin workflows • Scalable production from milligram-level R&D batches to multi-gram lots • Specialized purification workflows designed to achieve endotoxin levels below quantification limits (typically <0.5 EU/mg or lower depending on project needs) • Various chromatography (affinity, IEX, SEC) for different design of proteins	
	Purity & Identity	• SDS-PAGE under reducing/non-reducing conditions • SEC-HPLC with high-resolution quantification of monomer/aggregate profiles • Western blot confirmation (optional) • LC-MS for intact mass analysis (optional)
	Binding & Function	• Bio-Layer Interferometry (BLI) for affinity and kinetics • ELISA-based activity assays or custom bioassays (cell-based or biochemical)
	Endotoxin Tests	• rFc endotoxin quantification
DataPackage & Deliverables	• Fully traceable batch record and QC summary report. • Certificates including purity, concentration, endotoxin level, and method descriptions. • Customized formulation (buffers, excipients, stabilizers) suitable for immunology, vaccine research, or cell therapy tool development.	

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