

Use of Pharmaceutical Grade **Human Thrombin** in the Manufacture of Plasma-Derived Serum (PDS) for Cell Culture Applications

BioSupplies

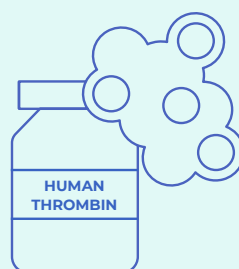
Biological material solutions

Cell Therapy

Application Note

Human serum—particularly male AB serum—is a critical ancillary material for the manufacturing of cell and gene therapy products. Its unique composition of growth factors, cytokines, hormones, lipids, and nutritional proteins supports cell proliferation, viability, differentiation, and therapeutic potency. Male AB donors are preferred due to the absence of anti A and anti B antibodies and the lower likelihood of HLA antibodies, minimizing immunogenicity risks in downstream therapeutic use.

Traditionally, the highest performing serum for cell culture has been off the clot (OTC) serum, obtained by allowing whole blood to clot naturally. However, global whole blood donation volumes are insufficient to meet the rapidly growing demand generated by cell therapy programs. As a result, large scale, consistent, safe serum production requires the use of plasmapheresis-derived plasma, converted into serum via a controlled defibrination process.



THE ROLE OF HUMAN THROMBIN

To convert plasma into serum, fibrinogen must be removed, which requires the enzymatic action of thrombin. Thrombin (Factor IIa) cleaves fibrinogen into fibrin monomers, allowing a stable clot to form and be removed. This process replicates physiological clot formation and yields serum functionally comparable to OTC serum.

Grifols exclusively uses in-house manufactured, human pharmaceutical-grade thrombin (xeno-free) to ensure the safety of plasma-derived human male AB serum.

KEY REASONS TO USE HUMAN THROMBIN

	HUMAN THROMBIN (GRIFOLS)	BOVINE THROMBIN
Origin	Human plasma	Animal-derived (cow)
Xenogenic risk	None	Significant (animal proteins, BSE concerns)
Regulatory acceptance for human cell therapies	High (aligned with pharmacopeial standards)	Low (Xeno-free requirements)
Compatibility with human cells	Native human enzyme	Possible cross reactivity or immunogenicity

For these reasons, regulatory agencies and cell therapy developers strongly prefer xeno-free ancillary materials, making pharmaceutical grade human thrombin a highly suitable and compliant choice.

GRIFOLS

MECHANISM OF ACTION

Human thrombin is a serine protease with key roles in coagulation and cellular signaling:

1. Fibrin formation (defibrination)

- Converts fibrinogen → fibrin monomers
- Requires calcium ions for clot stabilization
- Activates Factor XIII → cross linked fibrin → removable clot

2. Exhibits OTC like serum characteristics

Human thrombin initiates fibrinogen cleavage and clot formation, but the resulting serum resembles naturally clotted serum only after excess calcium and residual anticoagulants are removed through filtration and dialysis, followed by pH adjustment.

BENEFITS OF USING HUMAN MALE AB SERUM (PLASMA-DERIVED SERUM)

Plasmapheresis as a source material offers clear advantages:

- Frequent plasmapheresis donations (up to twice weekly) support continuous, scalable collection and high plasma availability
- Controlled donor pool sizes (≤16 donors), in line with EU and US regulatory guidance
- Low lot-to-lot variability when carefully pooled and fully characterized

As a result, plasma-derived serum (PDS) manufactured using human thrombin provides:

- A reliable and globally scalable supply of human male AB serum
- Regulatory alignment for use as an ancillary material
- Consistent performance in cell expansion, viability, and potency

Due to the limited availability of OTC human serum, Plasma Derived Serum (PDS) has become essential for meeting global demand in cell therapy manufacturing. The use of pharmaceutical grade human thrombin is the safest, most regulatory aligned method to convert plasma into high quality serum while maintaining xeno free status and maximizing compatibility with human cell systems.

Grifols' integrated fractionation expertise and clinical grade thrombin manufacturing ensure a consistent, safe, and scalable supply of male AB serum suitable for research, clinical manufacturing, and commercial cell therapy processes.

SAFETY AND QUALITY MEASURES

Grifols integrates high quality standard protocols into all serum products. Together, these measures ensure that Grifols Male AB Plasma Derived Serum is fully aligned with the expectations for advanced therapy manufacturing.



HIGH STANDARD MATERIALS

Human, clinical grade thrombin (GMP manufactured) for consistent, reliable defibrination of the plasma.



TRACEABILITY AND REGULATORY COMPLIANCE

Full traceability and donor screening aligned with plasma derivative standards. Comprehensive quality documentation supporting regulatory needs.



MANUFACTURING

Manufactured in ISO-certified, cGMP facilities.



VIRAL RISK MITIGATION

Adequate multilayer viral risk mitigation strategy for ancillary material use in CT: Donor selection, plasma testing, and complementary viral inactivation/reduction measures (e.g., gamma irradiation).¹

