Lyset™ Kit

Cell Culture Supplement

Technical data sheet

ORDERING INFORMATION

Code	Composition
REF CSI087320	**Lyset™ -PL**: n° 1 vial x 5 ml (lyophilized) **Lyset ™ -AD**: n° 2 vials x 10 ml (lyophilized)

INTRODUCTION

Lyset is a sterile freeze-dried human platelet lysate, a blood derived product which is prepared from platelets isolated from Platelet Rich Plasma (PRP). Platelets are lysed within 2 days from collection and the platelet content is supplemented with anti-coagulant, processed, dispensed in vials, freeze-dried and stored at -20°C,
The bioactive factors, named Growth Factors (GF) and contained in the platelets, modulate different biological functions playing a role in the wound healing process, including cell chemo-attraction, cell proliferation, extracellular matrix synthesis, cell recruitment and induction of angiogenesis. The platelet lysate medium demonstrated lack of microorganisms, mycoplasma and endotoxins.

PRINCIPLE OF METHOD

The human Platelet Lysate (hPL) is being utilized to replace animal and human serum such as Fetal Bovine Serum (FBS) or Fetal Calf Serum (FCS) in culture medium formulation for different types of cells. The platelet lysate was investigated in cell culture studies (cell growth, viability and product release) towards a number of target cells including myelomas, hybridomas, fetal and adult stem cells, hepatocytes, fibroblasts, chondrocytes and epithelial cells. In general the platelet lysate medium supported cell growth and maintained viabilities superior to fetal bovine serum. For some types of cells, including adult stem cells, hPL is particularly effective in enhancing cell proliferation maintaining their differentiation potential.

REACTIVES

Lyset™ -PL : human platelet lysate from Platelet Rich Plasma
Lyset ™ -AD: human Platelet Poor Plasma

Storage and stability

= Storage temperature -20 °C

Store Lyset™ -PL and Lyset ™ -AD at -20°C before reconstitution. Lyset™ -PL and Lyset ™ -AD can be stored at -20°C until the expiration date printed on the label maintaining at least 80% of their initial activity. If strictly necessary the reconstituted products can be stored at 4°C up to 7 days or at -20°C (after aliquoting) for a maximum of 60 days. Mix well the solution before use. Repeated freeze and thaw cycles should be avoided.

REAGENT PREPARATION

Lyophilized reagents. The cell supplement is obtained by the combination of two components: Lyset™ -PL and Lyset ™ -AD .

1. Allow lyophilized supplement to equilibrate at room temperature.
2. Using aseptic techniques restore the content of the Lyset™ -PL vial with 5 ml of sterile distilled water.
3. Using aseptic techniques, restore the content of the Lyset ™ -AD vial with 10 ml of sterile distilled water.
4. After water addition, shake gently and wait 2-5 minutes for complete reconstitution; the final solutions are opalescent and may contain small aggregates. The presence of small aggregates in the culture medium does not interfere with culture conditions.

The lyophilized Lyset™ -PL product, restored with H_2O, has a platelet equivalent concentration of about $1x10^7$/µl. In a standard preparation of restored product the PDGF-BB (Platelet Derived Growth Factors) and VEGF (Vascular Endothelial Growth Factor) concentrations (quantified by Elisa assay) are at least 100 ng/ml for PDGF-BB and at least 2 ng/ml for VEGF.
The lyophilized Lyset ™ -AD restored with H_2O has a platelet equivalent concentration of less than $5x10^4$/µl.

Note: It is advisable to use product and diluent immediately after reconstitution.

USAGE

When used as cell culture medium supplement, the reconstituted Lyset™ -PL and the reconstituted Lyset™ -AD should be added to the basal medium in different ratio according to the cell type to be cultured. Concentration in the culture medium of the combined Lyset™ -PL + Lyset ™ -AD could vary according to cell type and experimental conditions.

The culture medium could be filtered after the addition of Lyset™ -PL supplement.
It is recommended that each user determines the optimal component ratio and the optimal concentration in the medium for the culture of the cells of interest.

Table 1. Example: 5% final concentration of the reconstituted and combined product Lyset™ -PL and Lyset ™ -AD in 100 ml of final medium

Lyset™ -PL (ml)	Lyset ™ -AD (ml)	Basal medium (ml)	Combination
5	0	95	A
2.5	2.5	95	B
1	4	95	C
0.5	4.5	95	D

Table 2. Example: 10% final concentration of the reconstituted and combined product Lyset™ -PL + Lyset ™ -AD in 100 ml of final medium

Lyset™ -PL (ml)	Lyset ™ -AD (ml)	Basal medium (ml)	Combination
1	9	90	E
0.5	9.5	90	F
0.2	9.8	90	G

Suggested starting combinations for primary cultures are combinations B and C.
Suggested starting combinations for cell lines are combinations D, E and F.

To passage the cells treat the cultures with trypsin as you would normally do when cells are grown in medium with conventional supplement. However, it is advisable to use Trypsin inhibitor, such as Trypsin Soybean inhibitor, after detachment of the cells with trypsin.

TRACEABILITY AND PRECAUTIONS

This product is for EXPERIMENTAL AND RESEARCH USE ONLY. The product may not be used in humans, as drug, agricultural or pesticidal product, food additive.
Normal human leukocyte and platelet rich blood fraction (buffy coats) from healthy donors are tested and found negative for HBV, HCV, HIV (both by serological and molecular biology tests).

Bibliography

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