Product Profile

Product Name:	*Cell-Qualified 0.1%Gelatin Solution*
Catalog Number	*01-944-1*
Unit Size Availability:	(B)100ml
Formulation:	Liquid Solution
Defined Storage Conditions:	2-8°C
Stability: (Under Defined Handling & Storage Conditions)	Please Refer To Product Label

Important Note! Please read the *MSDS* and *Product Profile* carefully in their entirety *before* using this material for possible safety precautions and potential hazards.

Product Description:

Attachment factors are structural proteins or protein-like substances that have adherent capabilities and increase cell-substrate interactions in a culture dependent attachment milieu. A number of glycoproteins have been identified that promote and/or influence in *vitro* cell attachment to the surface or substratum of the culture vessel. Normal attachment, growth and development of many cell types are dependent on attachment factors and extracellular matrix components. While some cells are able to synthesize these components, others require an exogenous source, particularly when grown in serum-free culture.

Often the growth and differentiation of anchorage-dependent cells are strongly influenced by either glass or plastic culture flasks utilized as a substrate. In order to facilitate attachment, cell spreading, growth, morphology, differentiation, and motility of your cells *Biological Industries* offers an extensive line of attachment and matrix factors. Each lot is cell culture tested to assess its ability to promote cell attachment and spreading. Gelatin is a heterogeneous mixture of water-soluble proteins of high-average molecular weights, present in collagen. Cell-Qualified 0.1% Gelatin Solution is intended for coating cell culture flasks or plates used for the growth of Mouse ES cells without a feeder layer as it improves cell attachment for certain types of primary cells as well as certain continuous cell lines. This sterile-filtered solution contains 0.1% Porcine gelatin in water.

One of the drawbacks in growing cells *in vitro* using conventional tissue culture techniques is that the cells rest on plastic rather than on their natural biological support. This natural support is a complex network of macromolecules known as the extracellular matrix or ECM. In broad terms, ECM has three major components: *Fibrous Elements* (e.g., Collagen, Elastin and Reticulin), *Link Proteins* (e.g. Fibronectin, Laminin) and *Space-Filling Molecules* (e.g. Glycosaminoglycans). ECM is produced by cells into the surrounding medium and it is strikingly vivid that the ECM can and does markedly influence the behavior of cells especially when growing cells *in vitro*. ECM holds cells and tissues together and provides a highly organized lattice within which cells can migrate and interact with each other. The matrix plays an active and complex role in regulating the behavior of cells that are in contact with it, influencing their shape, migration, proliferation and metabolic functions. In contrast, cells grown on plastic lose many of their natural differentiated properties due to the lack of interaction with ECM.

Collagen is a major structural protein of extracellular matrix and is the principal protein found in connective tissues. It is found not only in the organic portion of bones, skin, teeth and tendons but also occurs in other parts of the body as fibrous inclusions. Like other fibrous proteins, collagen is not readily available unless it undergoes heat treatment such as boiling which converts collagen into gelatin. It is an unusual protein rich in such amino acids as glycine, lysine, proline and others but unfortunately, not enough of the essential amino acids. Usually, the gelatin derived from collagen is a relatively poor-quality protein.

Some advantages include rapid attachment, high plating and cloning efficiencies, good proliferation, high saturation density, lower requirements for serum and added growth factors, better response to physiologically occurring hormones, expression of differentiated functions, cells have longer life span, flattening and morphological changes, and improved plating consistency.

Among the cells types showing a favorable response to ECM are Human, Bovine, Porcine and other cells of Mammalian origin.

For instance, Mouse Embryonic Stem Cells (MESC's) are used to generate mouse mutants by gene targeting and blastocyst-mediated transgenesis. Undifferentiated ES cells may be maintained *in vitro* for extended periods without loss of differentiation capacity when re-implanted back into a blastocyst. Well-established general culture conditions usually require the undifferentiated ES cells to be grown on inactive feeder cell layers or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium to influence cell growth and function. Growth and differentiation of anchorage-dependent cells are strongly influenced by glass or plastic cultureware offered as a cell-substrate interactive platform. Cell growth rates may be exponentially improved by specialized surface treatments or coating with attachment factors such as Gelatin Solution with LIF. Leukemia Inhibitory Factor (LIF), a pleiotropic, polyfunctional glycoprotein(IL-6) cytokine, should be added to the medium which impacts growth promotion and prevents cell differentiation on a wide array of various tissue types and target cells.

General culture conditions are now well established and thus require ES cells to be grown on an inactive feeder-cell layer or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium as aforementioned. Mouse ES basal medium has been optimized to grow and maintain undifferentiated mouse embryonic stem cells. The medium may be used with the addition of Fetal Bovine Serum (FBS) or with any other serum replacement designed for mouse ES cells. The medium also contains for your convenience, a stable glutamine dipeptide with a relatively long shelf-life when handled and stored properly under defined conditions of 2-8°C.

Predominant Characteristics of Cell-Qualified 0.1%Gelatin Solution include:

- § Optimized to Grow and Maintain Undifferentiated Mouse ES Cells
- § Animal Derived
- § Meets USP and EPTesting Specifications
- § Sterile-Filtered and Cell-Culture Tested
- § Suitable for Cell-Culture & Molecular Biology Applications
- § Long-Storage When Handled and Stored Properly Under Defined Conditions

Storage & Stability:

This product should be stored under specified conditions and used within the time frame specified on the label. Do ***not*** use after the expiration date as specified on the label. ***Deterioration of liquid media*** may be recognized by any of the following characteristics, among others including: (a). color change, (b). granulation/ clumping, (c). insolubility, (d). And/or decrease in expected performance parameters. Any material described above should not be used and therefore discarded. Do not use if a visible precipitate is observed in the medium. It is recommended that the Sterile-filtered Gelatin Solution should be kept at 2-8ºC. Store the medium in the dark as it is light sensitive. Stability: Two (2) years from date of production. Sterile solutions stored under specified conditions are stable indefinitely. However at elevated temperatures, hydrolysis or rupture of peptide bonds occurs thereby increasing the number of free amino groups. At higher temperatures and/or prolonged heating, there is a tendency in which gel strength and viscosity will be substantially degraded. Other notable factors influencing degradation are proteolytic enzymes, pH extremes and bacterial action.

As the selection of a nutrient medium or supplementation thereof is strongly influenced, among others, by many factors, of note are three major considerations:

- ◊ Cell Type
- ◊ Type of Culture(e.g., Clonal, Monolayer, Suspension)
- ◊ Degree of Chemical Definition

It is recommended to review the extensive literature concerning cell-culture media and its supplementation and the physiological parameters required for each specific cell-line as per their essential requirements.

Instructions/Procedure:

Recommended Procedure for Using Cell-Qualified(0.1%)Gelatin Solution

1) *Before* use, warm the Gelatin Solution to Room Temperature (15-30°C).
2) Using aseptic technique, add a sufficient amount of Gelatin solution to cover the bottom of the plastic ware(i.e.2ml/15cm² area).
3) Leave the Gelatin Solution in the plates/wells at Room Temperature or at 37°C in the incubator for 30-60 minutes.
4) Remove the Gelatin Solution by aspiration technique just prior to adding the medium and Mouse ES cells.
5) Important Note: The Gelatin-coated plates can be stored in sterile packaging at room temperature for up to one week. Prevent the plates from drying out!

Please Note: This medium can be used with feeder-layer dependent ES cell lines or with feeder-independent ES Cell Lines using Gelatin-Coated Plates.

Quality Control

Test	Specifications
Appearance/Description:	Clear ,Amber Colored Solution
Cell-Culture:	Pass
Osmolality:	275-300mOsm/kg
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
Mouse ES Basal Medium	01-171-1	-20°C
Dulbecco's Phosphate Buffered Saline(DPBS) without Calcium and Magnesium	02-023-1	Room Temperature(15-30°)
L-Glutamine Solution 29.2mg/ml in Saline	03-020-1	-20°C
L-Alanyl-L-Glutamine Solution(A Dipeptide Substitute)	03-022-1	-20°C
Penicillin-Streptomycin Solution,10,000 units/ml Penicillin G Sodium Salt,10mg/ml Streptomycin	03-031-1	-20°C
Fetal Bovine Serum	04-001-1	-20°C
Fetal Bovine Serum(Qualified for Human Embryonic Stem Cells)	04-002-1	-20°C
Crystalline Trypsin Solution	03-047-1	-20°C
Sterile Culture-Grade Water	03-055-1	Room Temperature(15-30°)
Cell Dissociation Solution, Non-Enzymatic	03-071-1	2-8°C
Serum-Free Cell Freezing Medium	05-065-1	2-8°C
L-Arginine Hydrochloride Cell Culture Grade Biochemical Formulation	41-201-	Room Temperature(15-30°)
Note: For a list of other Antibiotics, Serum, Reagents and Supplements, please refer to our Product Catalog, Product Profiles, Product Guides and Internet Site.		

References:

[1] O'Neil Maryadele *et. al*., The Merck Index, 14th Edition, Whitehouse Station, New Jersey,2006
[2] Lackie, J. M. The Dictionary of Cell & Molecular Biology, Academic Press: London, 2007
[3]Nussbaum, Robert L. *et. al*., Thompson & Thompson: Genetics In Medicine. W. B. Saunders: Philadelphia, 2001