



Biological Industries Israel  
Beit Haemek Ltd.

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### Product Profile

Product Name:	Potassium Chloride 0.075 M Solution
Product Catalog Number	12-005-1
Concentration:	0.075 M
Unit Size Availability:	(B) 100ml
Formulation:	Clear, Colorless Solution
Specified Storage Conditions:	2-8°C
Stability: (Under Specified Handling & Storage)	Please Refer to the 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

*Potassium Chloride 0.075 M* is a hypotonic solution, utilized for the preparation of blood lymphocyte chromosomes. Hypotonic treatment with KCl, often with the addition of Sodium Citrate is used most often to enhance membrane permeability and induce hypotonic cell swelling at either room temperature (15-30°C) or 37°C. Usually, the higher temperature is used to increase metaphase spreading. There are several factors that influence the extent of the hypotonic treatment utilizing KCl and/or Sodium Citrate:

- ◆ Cell Density
- ◆ Specimen Type (e.g. slides or cell pellet)

It is important to note that the type, extent and application of hypotonic treatment is determined for the most part, empirically, in the laboratory by trial and error and therefore may need to be modified from time to time to meet particular requirements and/or applications.

### Metaphase Spread

The Metaphase Spread is the beginning of chromosomal analysis, most often a very labor-intensive procedure in which cells derived from amniotic fluid, blood, bone marrow, chorionic villi or other tissues are grown in cell culture. The evident and random distribution of chromosomes must be displayed in an easily defined arrangement or format. In order to effectively and efficiently evaluate and analyze the number and structural normality of each chromosome, the process of pairing them takes place by segueing into a karyotype which is an orderly arrangement of the metaphase spread of chromosomes containing thousands of genes that are stored in DNA.

### Hypotonic Treatment

When evaluating Peripheral Blood Lymphocyte cytogenetic changes such as detecting karyotype aberrations and/or recurring structural chromosome abnormalities, chromosome preparations especially from actively dividing cell cultures do not always produce a rewarding metaphase spread or yield and in such cases, hypotonic treatment is often thought to be a most relevant factor for an acceptable outcome. This is where KCl and/or Sodium Citrate come into play. After the lymphocytes are grown in culture and then subsequently cultured again in a medium of choice with PHA-M and 10% FBS, they may be harvested with or without Colcemid Solution depending upon the method utilized. Afterwards they are subject to a hypotonic treatment varying in length relative to work protocols. The hypotonic treatment application is used to enlarge cells for adequate spreading of the metaphase chromosomes. Afterwards, Fixation and/or Slide Preparation complete the process.

### Peripheral Blood Lymphocytes

Peripheral blood lymphocytes also known as PBL's are mature lymphocytes that circulate in the blood rather than localizing in organ systems such as the spleen or lymph nodes. In its normal environment and in most vertebrates, a cell is bathed in a fluid similar to its contents. So if the flow within and without the cell are about equal, this is known as an isotonic or isoosmotic solution where, in this case, no net flow movement may occur. However, if the cell is placed within a new environment such as in culture media, far-reaching changes may occur if the *in vitro* environment does not mimic the *in vivo* environment to the nth degree. However in cell culture, we artfully create situations to meet our requirements.

In some appropriate hypotonic solutions, cultured mammalian cells may rapidly increase in volume and then shrink gradually over a relatively short-period of time within 20-30 minutes at 37.5°C. However, for example, an animal cell placed in a hypertonic solution (i.e. which has a higher osmotic pressure) than the cell cytoplasm will lose intracellular fluids to the surrounding environment and shrink or contract.

If we take another example in contrast to the aforementioned, an animal cell such as a lymphocyte placed in a hypotonic or perhaps a hyposmotic environment would be unable to maintain its equilibrium and thus lose its shape because it will gain water (i.e. more water will flow into the cell than out of it). In this case, the pressure or concentration inside the cell is greater and the solvent would continue to diffuse across the cell membrane causing the cell to eventually burst. The rupture of any cell by a hypotonic solution is known as plasmolysis, if however it occurs within an erythrocyte or RBC it is known as hemolysis, whereas the shrinking or shriveling of the inside of the RBC is known as crenation.

Generally in hypotonic solutions, there is a lower concentration of solute outside a cell, creating an environment with a lower osmotic pressure than that contained within the cell itself. The body would then attempt to achieve equilibrium across the cell membrane in an effort to compensate for or to equalize the concentration or the osmotic pressure which is a measure of the tendency of a solution to take in water by osmosis.

The solute to solvent concentration determines the solution tonicity which is important with respect to membrane permeability and diffusion. We see that most vertebrates are either hypotonic or hypertonic relative to their environment and therefore tend to gain or lose fluids according to physiological mechanisms. These mechanisms assist most vertebrates in maintaining not only a constant blood osmolality but also relatively fixed concentrations of individual ions across concentration gradients.

*Some Predominant Characteristics of Potassium Chloride 0.075M Solution include:*

- § Sterile & Easy-To-Use
- § Increases Metaphase Spread of Peripheral Blood Lymphocytes(PBL's)
- § Has Very Similar Properties to its Relative NaCl

**Storage, Handling, Stability Precautions and Disclaimer:**

For *in vitro* diagnostic use only. It is not intended for therapeutic use.

*Potassium Chloride 0.075M Solution* is stable when stored under specified conditions of 2-8°C unopened until the expiration date appearing on the label. The KCL may also be light-sensitive so store at the specified conditions stipulated and preferably in the dark.

As with any other liquid media formulations, deterioration of liquid media may be recognized by any of the following characteristics, among others including: (a). color change, (b). presence of clumping/flocculent debris/ granulation/ particulates\ precipitates or sediments (c). insolubility,(d). and/or decrease in expected performance parameters. Any material described above should not be used and therefore discarded.

Although each batch of *Potassium Chloride(KCl) 0.075M Solution* is tested for performance, the use of Biological Industries *KCl* does not guarantee the successful outcome of any diagnostic testing.

#### *Instructions/Procedure*

- 1) Take a bottle of *Potassium Chloride(KCl) 0.075M Solution* from the specified storage conditions at 2-8°C and read the label.
- 2) Wipe the outside of the bottle with a disinfectant solution such as 70% ethanol.
- 3) Using aseptic/sterile technique under a laminar-flow culture hood and work according to established protocols.

#### *Quality Control*

Test	Specification
Appearance:	Clear Solution
Osmolality:	130-140 mOsm/kg
Sterility:	Sterile

**Auxiliary Products**

Product Name	Catalog Number	Storage Temperature
BIOAMF-1 Basal Medium	01-190-1	2-8°C
BIOAMF-1 Supplement	01-192-1	-20°C
BIOAMF-2 Complete Medium	01-194-1	-20°C
BIOAMF-3 Complete Medium	01-196-1	-20°C
Peripheral Blood Karyotyping Medium Without Phytohemagglutinin	01-198-1	-20°C
Bone Marrow Karyotyping Medium Without Conditioned Medium	01-199-1	Please See Product Label
Hematopoietic Cell Karyotyping Medium With Conditioned Medium	01-200-1	-20°C
EZ Lympho-Sep™-Lymphocyte Separation Tubes	01-899-U	Please See Product Labels
Sodium Citrate Solution (0.8%)	01-934-1	Room Temperature(15-30°)
Trypsin EDTA,10X Concentrate	03-051-1	-20°C
Colcemid Solution, 10µg/ml in DPBS	12-004-1	2-8°C
Potassium Chloride(KCl) Solution(0.075M)	12-005-1	2-8°C
Phytohemagglutinin-M(PHA-M)	12-006-1	2-8°C
Cell Synchronization Kit	12-008-60	-20°C
<b>Note:</b> For a list of Antibiotics, Serum, Reagents and other Supplements, please refer to our Product Catalog/Product Profiles/Product Guides and Internet Site.		

**References:**

- 1) Moorehead, P.S. *et al.*, "Chromosome Preparations of Leukocytes Cultured from Human Peripheral Blood," *Ex. Cell Res.*, 20:613-616(1960).
- 2) Nowell, P.C. "Phytohemagglutinin- An Initiator of Mitosis in Cultures of Normal Human Leukocytes," *Cancer Research*, 290:462-466(1960).
- 3) Barch, M.J.(ed.). The Association of Cytogenetic Technologists Laboratory Manual, Second Edition, (1991).
- 4) Biological Industries (BI) Specifications
- 5) Martindale, *The Extra Pharmacopeia*, 28<sup>th</sup> Edition, Royal Pharmaceutical Society: London, England pps.416-417.
- 6) Biological Industries (BI) *Product Guide*, "Human Cytogenetic Products," p.8.
- 7) O'Neil, MaryAdele J. *et al.*, *The Merck Index*, 14<sup>th</sup> Edition. White House Station, New Jersey, 2006, Monograph Number: 2471, p.2476