M2 MEDIUM
Without Penicillin, Streptomycin, Lactic Acid and Sodium Bicarbonate

Product Number **M5910**
Storage Temperature 2-8°C

ProductInformation

Product Description

M2 medium, a variation of M16 medium containing HEPES in place of some of the sodium bicarbonate, is used to collect mouse embryos and to handle them for extended periods of time outside of the incubator. Embryos are extremely sensitive to variations in the culture medium, much more so than other cells or tissues.

M2 MEDIUM, Product No. M5910 is one of the cell culture media available from Sigma. The selection of a nutrient medium is strongly influenced by 1] type of cell, 2] type of culture [monolayer, suspension, clonal] and 3] degree of chemical definition necessary. It is important to review the literature for recommendations concerning medium, supplementation and physiological parameters required for a specific cell line.

Components	g/L
Calcium Chloride• $2H_2O$	0.25137
Magnesium Sulfate [anhydrous]	0.143276
Potassium Chloride	0.356349
Potassium Phosphate Monobasic	0.161959
Sodium Chloride	5.5319304
Albumin, bovine fraction V	4.0
Glucose	1.001912
Phenol Red•Na	0.010620767
Pyruvic Acid•Na	0.0363
HEPES Free Acid	4.96855

M2 normally contains the indicated concentrations of the following components:

	g/L
Penicillin G Potassium	0.06
Streptomycin Sulfate	0.05

Precautions and Disclaimer

REAGENT
For Laboratory Use Only.
Not for Drug, Household or Other Uses.

Preparation Instructions

Powdered media are extremely hygroscopic and should be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. Preparing a concentrated solution of medium is not recommended as precipitates may form.

Supplements can be added prior to filtration or introduced aseptically to sterile medium. The nature of the supplement may affect storage conditions and shelf life of the medium.

1. Measure out 90% of final required volume of water. Water temperature should be 15-20°C.
2. While gently stirring the water, add the powdered medium. Stir until dissolved. Do NOT heat.
3. Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
4. To the solution in step 3, add 4.349 g of DL-Lactic Acid•Na [60% syrup] for each liter of final volume of medium being prepared. Stir until dissolved.
5. To the solution in step 4, add 0.35 g sodium bicarbonate or 4.65 ml of sodium bicarbonate solution [7.5%w/v] for each liter of final volume of medium being prepared. Stir until dissolved.
6. While stirring, adjust the pH of the medium to 0.1-0.3 pH units below the desired pH since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended.
7. Add additional water to bring the solution to final volume.
8. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
9. Aseptically dispense medium into sterile container.

Storage/Stability

Store the dry powdered medium at 2-8°C under dry conditions and liquid medium at 2-8°C in the dark. Deterioration of the powdered medium may be recognized by any or all of the following: [1] color change, [2] granulation/clumping, [3] insolubility. Deterioration of the liquid medium may be recognized by any or all of the following: [1] pH change, [2] precipitate or particulate matter throughout the solution, [3] cloudy appearance [4] color change. The nature of supplements added may affect storage conditions and shelf life of the medium. Product label bears expiration

date.

Procedure

Materials Required But Not Provided:
Water for embryo culture [W1503]
DL-Lactic Acid•Na [60% syrup] [L7900]
Sodium Bicarbonate [S5761] or
Sodium Bicarbonate Solution, 7.5% [S8761]
1N Hydrochloric Acid [H9892]
1N Sodium Hydroxide [S2770]
Medium additives as required

Product Profile

Appearance	off-white powder
Moisture content	≤ 2.0%
Solubility	clear solution at 1x concentration
pH at room temperature [without sodium bicarbonate]	5.9 ± 0.3
pH at room temperature [with sodium bicarbonate]	6.4 ± 0.3
Osmolality [without sodium bicarbonate]	231 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	245 mOsm/kg H_2O ± 5%
Endotoxin	≤ 10 EU/ml at 1x
Key Element Analysis by ICAP	Analysis has confirmed that key elements are present at concentrations consistent with the formula.

BIOLOGICAL PERFORMANCE CHARACTERISTICS
Product is tested for its ability to support the development of one-cell mouse embryos to expanded blastocysts. B6C3 F1 hybrid mice are used. The mice are superovulated with PMSG and hCG. Zygotes are collected 18-22 hrs after hCG injection, treated with hyaluronidase and cultured for 96-108 hours. Minimum requirement is 80% development to blastocyst.

References

1. Manipulating the Mouse Embryo A Laboratory Manual (1986) Hogan B., Costantini F., and Lacy E. eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
2. Whitten W. K. and Biggers J. D. (1968) J. Reprod. Fert. 17: 399-401.
3. Whitten W. K. (1971) Adv. Biosci. 6: 129-141.
4. Whittingham D. G. (1971) J. Reprod. Fert. 14: 7-21.

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