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Cell culture medium for in vitro research use only

S-Clone SF-O3

A serum-free medium for use in research of hematopoietic stem cells

[Development Background and Features]

S-Clone SF-O3 is a completely serum-free medium for use in research of mouse hematopoietic stem cells. The product was developed based upon the results of research by the Nishikawa Laboratory, Department of Molecular Genetics at the Graduate School of Medicine, Kyoto University. It is used for molecular studies on proliferation and maintenance mechanisms of mouse hematopoietic stem cells and has been reported effective in the same.

The medium is serum-free, which eliminates effects of any unknown factors in serum, and it contains extremely limited protein components, allowing the action of cytokines, etc. to be clearly identified. Further, being composed of known ingredients, the product affords constancy in experimental conditions. (See Reference Literatures: (1), (2), (3), (4), (5) , (6))

[Formulation]

1. Basal medium (powder)
   Constituted from a composite of slightly modified RPMI1640, Dulbecco's MEM, and Ham's F-12.

2. Sodium hydrogen carbonate (powder)

3. Supplement (liquid)
   HEPES buffer containing hormone growth factors (**rh-insulin, transferrin, sodium selenite and ethanolamine). Protein components other than insulin and transferrin have not been added.

[Directions for Use]

<Preparing the Medium>

1. Add 1 bottle of the basal medium (10.7 g) to approximately 950 mL of purified water and mix well to dissolve.

2. To the solution above, add 1 vial (2.2 g) of sodium hydrogen carbonate and dissolve, and further dilute to a total 1,000 mL.

3. Sterilize by filtering through a low adsorption membrane filter of pore size 0.2-0.22 μm (Toyo Roshi Kaisha Ltd., Cat. No. C020A047A or Millipore Corporation, Cat. No. GVWP04700, etc.).

4. Aseptically add 1 vial of the supplement (10 mL) to the filtered 1,000 mL of medium.

5. Seal and store refrigerated (2-10°C).

<Examples of Usage>

1. Method for cultivation of B precursor cells on a mouse stromal cell line ST2 layer using SF-O3 (Add IL-7: 10 U/mL, 2-mercaptoethanol: 100 μmol/L).

   1) Seed the ST2 cells at a density of 1.5×10^5 cells/well to make the feeder layer.

   2) Seed the mouse bone marrow cells at a density of 2×10^5 cells/well on the ST2 feeder layer and continue to incubate, replacing half of the medium every 4-5 days.

   Well used: Falcon Primaria™ Cultureware 6-Well Plate (3846)

2. Method for cultivation of hematopoietic precursor cells on a mouse stromal cell line PA6 layer using SF-O3 (Add bFGF or EGF: 5-10 ng/mL, 2-mercaptoethanol: 100 μmol/L).

   1) Seed the PA6 cells at a density of 2×10^5 cells/well to make the feeder layer.

   2) Seed the mouse bone marrow cells at a density of 2×10^5 cells/well on the PA6 feeder layer and continue to incubate, replacing half of the medium every 4-5 days.

   Well used: Falcon Primaria™ Cultureware 6-Well Plate (3846)

3. **Method for cultivation of mesendoderm cells to induce differentiation using SF-O3 (Add bovine serum albumin: 0.1%, 2-mercaptoethanol: 50 μmol/L as the differentiation induction medium).

   1) Suspend the undifferentiated mouse ES cells in the differentiation induction medium at a density of 1×10^4 - 3×10^4 cells/mL, then add activin at a density of 10 ng/mL.

   2) Seed 10mL of the cell suspension above to type-Ⅳ collagen coated cell culture dish of 10cm diameter makes a density of 1×10^5 - 3×10^5 cells/plate, and then culture for 4-6 days.

[Precautions for Use and Handling]

1. The entire bottle of basal medium should be dissolved in a single use. Avoid dissolving the powder in small portion.

2. When dissolving the basal medium, inject carbon dioxide gas (high-quality carbon dioxide or dry ice is suitable) to shift the pH of the medium to acidic. An acidic pH enhances solubility of the medium as well as speeds equilibration (pH 7.2-7.4) of the medium in the carbon dioxide gas incubator.

3. As the supplement is a concentrated solution, it may show precipitation when stored refrigerated. It should be mixed well before adding to the basal medium.

4. Avoid filtering the medium after the supplement has been added, as it may cause lowered growth in some types of cells.

5. The prepared medium is confirmed to be stable for 3 months when stored at 2-10°C, however, it should be used as soon as possible.

6. **There might cause some precipitates while storing the prepared medium. They are no hindrance to a performance, stir well and use as it is.

7. This product does not contain 2-mercaptoethanol.

8. This product is a reagent for in vitro research use only and should not be used for diagnostic purposes, nor should the grown cells be used for treatment.

9. Treatment of wastes generated by this method should be done according to guidelines in accordance with the local laws and regulations.

[Storage and Shelf Life]

- Store at 2-10°C, avoid freezing.
- **The shelf life is 2 year following manufacture (expiration date is indicated on the outer box)

[Package Contents]

- Basal medium: 1 L-equivalent × 1 bottle
- Sodium hydrogen carbonate: 2.2 g × 1 vial
- Supplement: 10 mL × 1 unit vial

[Reference Literature]

(1) Immunology Letters. **40**: 163-169, 1994
(2) Seminars in IMMUNOLOGY, **7**: 185-196, 1995
(3) J. Exp. Med., **182**: 315-323, 1995
(4) Clinical Immunology, **27(10)**: 1193-1198, 1995
(5) Journal of Cell Biology, Volume 132, Numbers 1&2, January 1996 91-99
(6) Development, 132：4363-4374, 2005

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