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ProductInformation

AMNIOCYTE MEDIUM, BPE-free

Product Code **A 6840**

Storage Temperature –20 °C

Product Description
Over the last 30 years, prenatal chromosome diagnostic testing has become an important technique in monitoring fetal abnormalities. The speed and accuracy of prenatal chromosome diagnostic testing is dependent on several factors; adequate specimen collection and proper storage, reproducible techniques for chromosome harvest and identification, and optimal cell culture conditions that provide a significant number of analyzable cells. Sigma's Amniocyte Medium, BPE-free is a highly optimized medium intended for the culture of human amniotic fluid cells (AFC) used in prenatal diagnostic testing.

Amniocyte Medium, BPE-free is a nutritionally complete medium requiring only the addition of antibiotics. It is composed of a basal medium and supplements. The product is supplied frozen and needs no additional supplementation.

Intended Use
For *In Vitro* diagnostic use.

Components
Basal Medium: Modified alpha-MEM
Buffers: HEPES, Sodium Bicarbonate
Serum: Fetal Bovine
Other Components
Growth factors and hormones

Storage/Stability
Amniocyte Medium, BPE-free should be stored in the dark at freezer temperatures (–20 °C). After thawing, medium should be kept at refrigerated temperatures (2-8 °C). DISCARD THE MEDIUM WITHIN 10 DAYS AFTER THAWING. Frost-free freezers and repeated freeze thaw cycles can accelerate product breakdown and should be avoided. Avoid exposure to light. Any or all of the following may be recognized as deterioration of the medium: [1] color change, [2] cloudiness, [3] pH change and [4] diminished cell growth and poor chromosome morphology. Label bears expiration date.

Procedure
1. Thaw medium at refrigerator temperatures (2-8 °C). Mix gently after thawing. Warm medium to 37 °C before use.
    1. Add antibiotics if desired. Gentamicin Solution (Sigma Product Code G 1272 or G 1397) is recommended. Penicillin/Streptomycin (Product Code P 0781 or

    P 4333) may be used after qualification by the laboratory.
3. A recommended protocol for the culture and harvest of amniocytes is given below. Detailed protocols for all of Sigma's Cytogenetics products are also available at Sigma-Aldrich's Web site: **sigma-aldrich.com**.

Recommended protocols for the culture and harvest of amniotic fluid cells:
The first step in setting up amniotic fluid specimens is to determine the type of vessel to be used to culture the amniocytes and the number of coverslips or flasks to be used. The general rule is to allow 5 ml of amniotic fluid per coverslip as indicated in the chart below.

Fluid Amount	Number of Coverslips
25-30 ml	6 coverslips
20-25 ml	5 coverslips
15-20 ml	4 coverslips
10-15 ml	3 coverslips
10 ml and under	2 coverslips

In–situ culture of amniotic fluid cells:
1. Between 15-30 ml of fluid is normally used to complete a full cytogenetics study. Less fluid may result in an increase in the amount of culture time.
2. Using sterile technique dispense the amniotic fluid into two 15 ml centrifuge tubes. Determine the number of cultures being set-up based on the chart above.
3. Centrifuge the tubes at 1,000 rpm for 10 minutes.

4. Carefully aspirate the supernatant from each pellet. If Alpha-Fetoprotein (AFP) or Acetylcholinesterase (AChE) studies are to be performed be sure to retain sufficient supernatant for these tests.
5. Leave approximately 0.7 ml of amniotic fluid supernatant above each pellet and mix with 0.7 ml of Amniocyte Medium, BPE-free (Product Code A 6840).
6. Resuspend each pellet in the supernatant/medium mixture.
7. Carefully transfer 0.7 ml of the cell suspension to each coverslip. Spread the cell suspension to cover the entire coverslip, being careful not to push the suspension off the edge of the coverslip.
8. After 48 hours flood each coverslip with 2 ml of culture medium. For example, if the cultures are being set-up on a Friday, the dishes may wait 72 hours before flooding, provided that there is at least 0.7 ml of suspension on each coverslip.
9. Change at least 50% of medium every other day until the cultures are ready to be harvested.
10. When the cultures have colonies of sufficient size, number and mitotic figures present, proceed with the harvesting.

Harvest of *In-situ* cultures:

1. Add 50 µl of Demecolcine (10 µg/ml, Product Code D 1925) to each culture dish being harvested. Incubate at 37 °C for 20 minutes.
2. Following incubation, aspirate the medium off each coverslip and gently add 2 ml of pre-warmed (37 °C) hypotonic solution such as 6:4 mixture of 0.6% Sodium Citrate hypotonic solution (Product Code S 4641) and 0.075 M KCL (Product Code P 9327).
3. Incubate the cultures at room temperature for 20 minutes.
4. Following incubation, add 1ml of Carnoy's fixative [75% methanol (Product Code M 3641): 25% Acetic Acid (Product Code A 6283)]. Let cultures stand for 2 minutes.
5. Aspirate the fluid off the dishes and gently add 2 ml of fresh Carnoy's fixative.
6. Let cultures stand for 10 minutes.
7. Aspirate the fixative and add 2 ml of fresh fixative.
8. Aspirate all the fixative off the coverslips and use a drying chamber or your laboratory's standard protocol for the drying of the coverslips.

Flask method culture of amniotic fluid cells:

1. Using sterile technique, dispense the amniotic fluid into two 15 ml centrifuge tubes.
2. Centrifuge the tubes at 1,000 rpm for 10 minutes.
3. Transfer the supernatant from each tube into separate T-75 flasks. Leave approximately 0.7 ml of supernatant above each pellet. If AFP or AChE studies are to be performed be sure to retain sufficient supernatant for these tests.
4. Add 5 ml of Amniocyte Medium, BPE-free (Product Code A 6840) to the T-75 flasks containing the supernatant. Place the caps loosely on the flasks and incubate at 37 °C and 5% CO_2.
5. Add 0.7 ml of culture medium to each of the pellets and resuspend by gentle mixing.
6. Transfer the cell suspension from each tube into separate T-25 flasks. Add 5 ml of culture medium to each flask.
7. Place the cap loosely on the flasks and incubate at 37 °C and 5% CO_2.
8. Check all flasks for growth in approximately 5-6 days.
9. When approximately 10-12 medium sized colonies are present, process the flasks using the protocol below.

Harvest of flask cultures:

1. Add 100 µl of Demecolcine (10 µg/ml, Product Code D 1925) to each flask and incubate for 1 hour.
2. Tap the flasks to get the cells in metaphase to float. Take off the medium and save it in 15 ml centrifuge tubes.
3. Rinse cells quickly with 2 ml of trypsin-EDTA (Product Code T 3924). Remove the trypsin-EDTA and add to the tubes with the aspirated medium.
4. Add 4 ml of fresh trypsin-EDTA to the flasks and incubate for 6 minutes.
5. Following incubation, add 5 ml of culture medium to the flasks to inhibit the trypsin action. Triturate the medium over the surface of the flasks to gently dislodge the cells. Take off the cell suspension mixture and place into separate 15 ml centrifuge tubes.
6. Centrifuge the tubes at 1,000 rpm for 10 minutes.
    1. Resuspend the cell pellets from each tube with 1 ml of the supernatant and combine the pellets into one tube. Fill the tube with 10 ml of pre-warmed (37 °C) Hypotonic Solution (Sodium Citrate 0.625%, Product Code S 4641).
8. Immediately centrifuge the tubes at 1,000 rpm for 10 minutes.
9. Resuspend the cells in 10 ml of hypotonic solution and incubate at 37 °C for 10 minutes.
10. Following incubation, add 8 drops of Carnoy's fixative [75% methanol (Product Code M 3641): 25% Acetic Acid (Product Code A 6283)] to the cell suspension, mix by inverting tubes, and centrifuge at 1,000 rpm for 10 minutes.
11. Aspirate the supernatant from each tube and resuspend the pellets in 10 ml of fresh fixative. Let cultures stand for 30 minutes.
12. Following incubation, centrifuge tubes at 1,000 rpm for 10 minutes.
13. Aspirate the supernatant from each tube and resuspend the pellets in 10 ml of fresh fixative.
14. Repeat steps 12 & 13.
15. Cell pellets can then be used immediately to drop slides according to your laboratory's standard protocol. Pellets may also be stored at 2-8 °C for future use.

Product Profile

Appearance	Clear solution.
pH at room temperature	7.4 ± 0.3
Osmolality	300 mOsm/kg H_2O ± 5%
Sterility by USP	Sterile
Endotoxin	≤5.0 EU/ml

AFC Performance Test	Pass

References

1. Barch MJ., Knutson T., Spurbeck, J.L., Eds. The ACT Cytogenetics Laboratory Manual, 3rd edition. 1997, New York: Raven Press
2. Rooney D.E., Czepulkowski B.H., Eds. Human Cytogenetics - A practical approach, 2nd Ed. Volume 1 - Constitutional Analysis, Oxford: IRL Press, 1992
3. Henry, G.P., Peakman, D.C., Robinson, A. Prenatal Genetic Diagnosis: Nine Years experience. Obstet. Gynecol. Survey **33**, 569 (1978)
4. Weaver, D. D. A survey of prenatally diagnosed disorders. Clin Obstet. Gynecol. Survey **31**, 253 (1988)

Precautions and Disclaimer

REAGENT

For *In Vitro* Diagnostic Use

1. Do not use if product is received thawed or shows signs of visible precipitate.
2. Do not dilute or mix this product with other media, alteration may result in negative effects on growth performance or chromosome integrity.
3. Product is not intended for therapeutic use.
4. Use of Sigma's Amniocyte Medium, BPE-free does not guarantee successful diagnostic procedures.

MSDS is available upon request at: **sigma-aldrich.com**.

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