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CHANG Amnio Recommended Protocol

Please refer to the Product Insert for detailed instructions and additional information.

PRODUCT DESCRIPTION

CHANG Amnio is a complete, ready-to-use medium for the primary culture of human amniotic fluid cells (AFC), chorionic villus sampling (CVS), and products of conception (POC) for use in karyotyping and other prenatal genetic testing. It has been optimized for both flask and *in situ* methodologies.

STORAGE AND STABILITY

Store frozen below -10°C. Product is stable until the expiration date on the bottle label when stored frozen. Unused product can be dispensed into working aliquots and refrozen for later use, or tightly capped and stored at 2–8°C for up to 30 days; it may be frozen a maximum of two times. Protect from fluorescent light.

PREPARATION FOR USE

Thaw rapidly by swirling bottle in a 37°C water bath.

CHANG Amnio contains gentamicin (50 mg/L). Additional antibiotics may be added if desired.

ALIQUOTING CHANG Amnio

1. Thaw CHANG Amnio according to the above instructions.
2. Distribute aseptically into convenient sized aliquots and refreeze.
3. Thaw aliquots in 37°C water bath when ready to use.

DIRECTIONS FOR USE

I. Primary Cultures: In Situ Methodologies

1. Centrifuge amniotic fluid at approximately 1, 200 rpm for 10 minutes to concentrate the cells.
2. Aspirate supernatant from the centrifuged tube, leaving approx. 0.5 mL above cell pellet (or about 2x volume of pellet) of spun amniotic fluid. Aliquot supernatant (at least 1 mL, if possible) for alpha-fetoprotein (AFP) and acetyl cholinesterase assays, if necessary. If specimen is bloody, prepare an additional aliquot for further testing.
3. Re-suspend cell pellet in a small volume of the patient's own amniotic fluid. Add sufficient CHANG Amnio to the concentrated cell suspension to allow for final plating volume of 0.5 mL per coverslip (total of 4 coverslips, depending on size of cell pellet) or 2 mL per flaskette. If the specimen is received from a patient in the third trimester of pregnancy, the pellet may be larger but contain less viable cells, thus requiring heavier seeding (less medium than normal).
4. Incubate cultures undisturbed at 37°C, 5–8% CO_2 atmosphere.
5. Flood cultures on day 2 by adding 2 mL of CHANG Amnio.
6. After 4 to 5 days, cultures should be checked for growth. Cultures should be fed once growth has been observed. Feed cultures by removing all of the culture supernatant and replacing with 2 mL fresh CHANG Amnio. It is recommended that cultures be fed every 2 days thereafter. For bloody specimens, cultures may require more frequent media changes.
7. Check cultures for growth on, or after, day 5, and harvest when sufficient colonies are observed.
8. Best results obtained when cultures are fed with CHANG Amnio the day before the harvest.

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II. Primary Cultures: Flask Methodologies

1. Centrifuge amniotic fluid at approximately 1,200 rpm for 10 minutes to concentrate the cells.
2. Aspirate supernatant from the centrifuged tube, leaving approx. 0.5 mL above cell pellet (or about 2x volume of pellet) of spun amniotic fluid. Aliquot supernatant (at least 1 mL, if possible) for alpha-fetoprotein (AFP) and acetyl cholinesterase assays, if necessary. If specimen is bloody, prepare an additional aliquot for further testing.
3. Re-suspend cell pellet in a small volume of the patient's own amniotic fluid. Add 4 mL of CHANG Amnio for a total volume of 5 mL per flask. If the specimen is received from a patient in the third trimester of pregnancy, the pellet maybe larger but contain less viable cells, thus requiring heavier seeding (less medium than normal).
4. Incubate cultures undisturbed at 37° C 5–8% CO_2 atmosphere.
5. Check for growth on day 5. Change medium with 2 mL of fresh CHANG Amnio and harvest if sufficient cell growth is observed.
6. Check cultures for growth and completely change medium every day thereafter until sufficient colonies are observed and are ready to harvest. For bloody specimens, cultures may require more frequent media changes.
7. Best results obtained when cultures are fed with CHANG Amnio the day before the harvest.

III. Growing Passaged Amniotic Fluid Cells

To passage the cells, treat cultures with trypsin (or pronase, etc.) as you would normally do when cells are grown in conventional medium. However, protease treatment should be carefully monitored. Amniotic fluid cells grown in CHANG Amnio tend to be more sensitive to protease treatment than when grown in conventional medium. It may be necessary to modify your protocol to take this into account.

The pH of the medium used to feed the cultures must be between 6.65–7.44 (i.e. the medium must be slightly yellowish salmon color). pH can easily be adjusted by placing the medium in a 5–8% CO_2 incubator with the cap slightly loosened for about 30 minutes.

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