CHANG Amnio Recommended Protocol for AFC

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

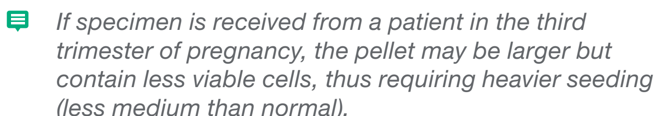
Thaw on a sterile countertop at room temperature or by placing bottle in a 37°C water bath.

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

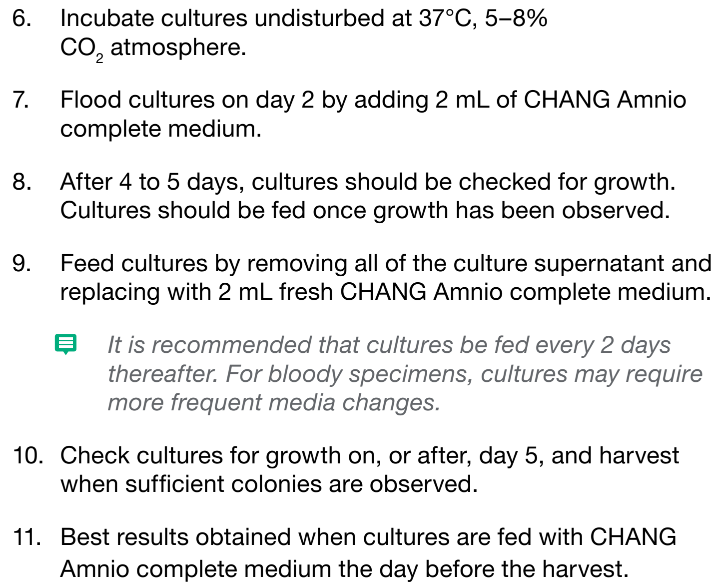
DIRECTIONS FOR USE

Amniotic Fluid Cells (AFC)
Primary Culture - In Situ Methodology:

1. Centrifuge amniotic fluid at approx. 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.
3. Aliquot supernatant (at least 1 mL, if possible) for alpha-fetoprotein (AFP) and acetyl cholinesterase assays, if necessary.
4. Resuspend cell pellet in a small volume of patient's own amniotic fluid.
5. Add sufficient CHANG Amnio complete medium 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 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).



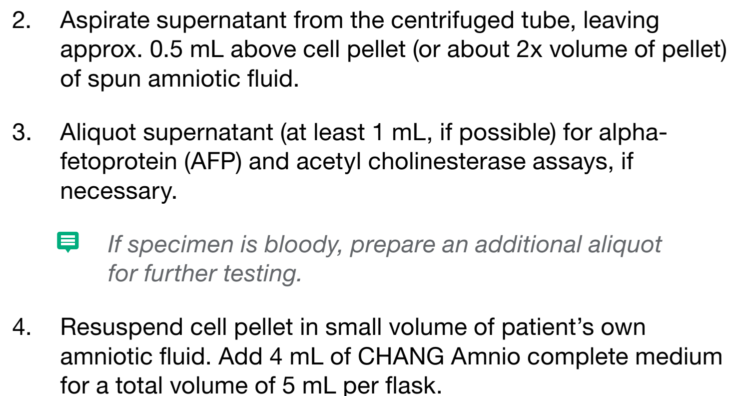
6. Incubate cultures undisturbed at 37°C, 5–8% CO_2 atmosphere.
7. Flood cultures on day 2 by adding 2 mL of CHANG Amnio complete medium.
8. After 4 to 5 days, cultures should be checked for growth. Cultures should be fed once growth has been observed.
9. Feed cultures by removing all of the culture supernatant and replacing with 2 mL fresh CHANG Amnio complete medium.

   It is recommended that cultures be fed every 2 days thereafter. For bloody specimens, cultures may require more frequent media changes.

10. Check cultures for growth on, or after, day 5, and harvest when sufficient colonies are observed.
11. Best results obtained when cultures are fed with CHANG Amnio complete medium the day before the harvest.

Amniotic Fluid Cells (AFC)
Primary Culture - Flask Methodology:

1. Centrifuge amniotic fluid at approx. 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.
3. 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.

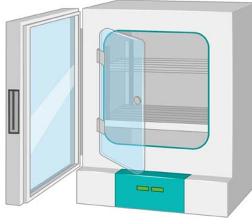
4. Resuspend cell pellet in small volume of patient's own amniotic fluid. Add 4 mL of CHANG Amnio complete medium for a total volume of 5 mL per flask.

CHANG Amnio Recommended Protocol for AFC

Continuation:

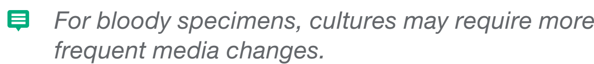
5. If the specimen is received from a patient in the third trimester of pregnancy, the pellet may be larger but contain less viable cells, thus requiring heavier seeding (less medium than normal).

6. Incubate cultures undisturbed at 37°C, 5–8% CO_2 atmosphere.



7. Check for growth on day 5. Change medium with 2 mL of fresh CHANG Amnio complete medium and harvest if sufficient cell growth is observed.

8. Check cultures for growth and completely change medium every day thereafter until sufficient colonies are observed and are ready to harvest.



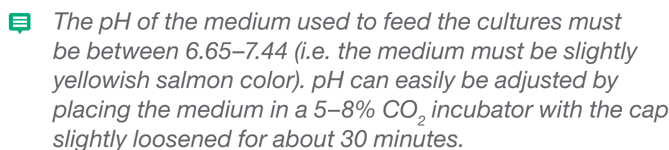
For bloody specimens, cultures may require more frequent media changes.

9. Best results obtained when cultures are fed with CHANG Amnio complete medium the day before the harvest.

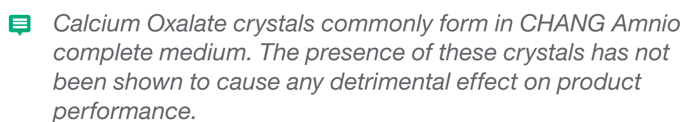
Amniotic Fluid Cells (AFC)
Primary Culture - Growing Passaged AFC

1. To passage the cells, treat cultures with trypsin (or pronase, etc.) as you would normally do when cells are grown in a conventional medium.

2. However, protease treatment should be carefully monitored. Amniotic fluid cells grown in CHANG Amnio complete medium tend to be more sensitive to protease treatment than when grown in a 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.



Calcium Oxalate crystals commonly form in CHANG Amnio complete medium. The presence of these crystals has not been shown to cause any detrimental effect on product performance.

Harvesting In Situ:

1. When coverslips are ready for harvest, use a sterile transfer pipette to add 1 drop of Colcemid (10 µg/mL). Incubate for 35 to 45 minutes at 35–39°C.

Do not place all of one patient's cultures in the same harvest, in case of harvest failure.

2. Remove all of the medium from the dish with a Pasteur pipette, working with one dish at a time.

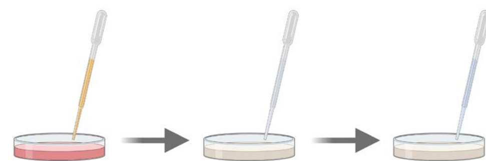
3. Slowly add 2 mL of prewarmed (35–37°C) sodium citrate hypotonic solution to the first dish, with a Pasteur pipette, being careful not to disturb the coverslip.

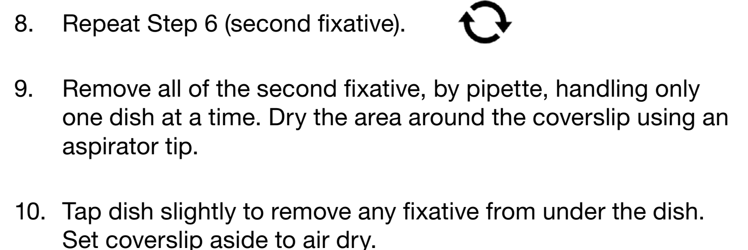
4. Repeat for all dishes being harvested. Leave for 25 minutes.

5. Add dropwise, 1 mL of fresh Carnoy's Fixative (3:1 Methanol: Glacial Acetic Acid) working with one dish at a time. Leave for 15 minutes.

6. Remove all of the hypotonic + fixative mixture, by pipette, then slowly add 2 mL of fresh fixative. Leave for 30 minutes.

7. Remove all of first fixative, by pipette, then slowly add 2 mL of fresh fixative. Leave for 20 minutes.





8. Repeat Step 6 (second fixative).

9. Remove all of the second fixative, by pipette, handling only one dish at a time. Dry the area around the coverslip using an aspirator tip.

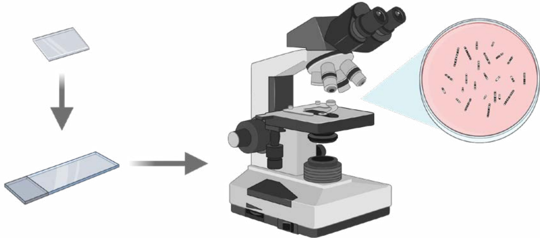
10. Tap dish slightly to remove any fixative from under the dish. Set coverslip aside to air dry.

CHANG Amnio Recommended Protocol for AFC

Continuation:

11. For best drying results, maintain a humidity of 45–65%, and temperature of 20–25°C.

12. When coverslip is dry, mount onto a microscope slide using a mounting medium, cell side up.

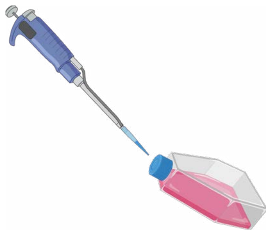


***Harvesting Flasks*:**

1. When flasks are ready for harvest, use a sterile transfer pipette to add 2 drops of Colcemid (10 µg/ml).

2. Prepare centrifuge tubes with a label that corresponds to each one of the flasks being harvested. Transfer contents of flask into the corresponding centrifuge tube.

3. Gently wash flask with 2 mL of prewarmed (35–37°C) Hanks' Balanced Salt Solution (HBSS 1X) and transfer to corresponding tube. Wash an additional two times using 2 mL of HBSS 1X each time.



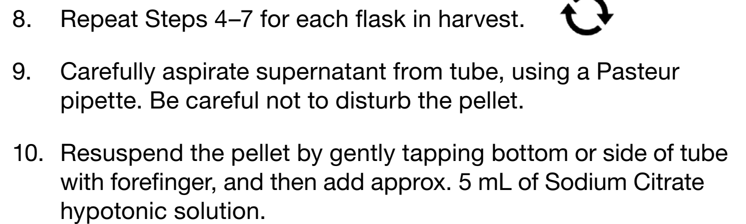
4. Add approx. 2 mL of Trypsin EDTA solution to the flask, and incubate for approx. 3 to 4 minutes.

5. Remove flask, after incubation period, and observe under inverted microscope to see if the cells are floating freely and no longer attached to the bottom of the flask.

6. Transfer contents of flask into corresponding centrifuge tube. Gently wash flask with prewarmed (35–37°C) HBSS 1X several times until tube is nearly full.

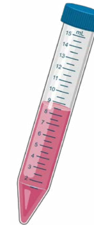
7. Centrifuge tubes at 1,000 rpm for 10 minutes to concentrate the cells.

8. Repeat Steps 4–7 for each flask in harvest.

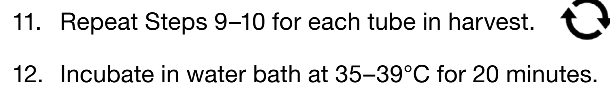


9. Carefully aspirate supernatant from tube, using a Pasteur pipette. Be careful not to disturb the pellet.

10. Resuspend the pellet by gently tapping bottom or side of tube with forefinger, and then add approx. 5 mL of Sodium Citrate hypotonic solution.



11. Repeat Steps 9–10 for each tube in harvest.



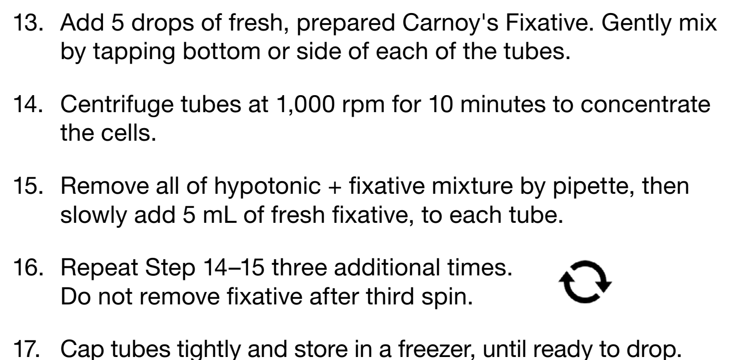
12. Incubate in water bath at 35–39°C for 20 minutes.

13. Add 5 drops of fresh, prepared Carnoy's Fixative. Gently mix by tapping bottom or side of each of the tubes.

14. Centrifuge tubes at 1,000 rpm for 10 minutes to concentrate the cells.

15. Remove all of hypotonic + fixative mixture by pipette, then slowly add 5 mL of fresh fixative, to each tube.

16. Repeat Step 14–15 three additional times. Do not remove fixative after third spin.



17. Cap tubes tightly and store in a freezer, until ready to drop.

CHANG Amnio Recommended Protocol for AFC

DISCOVER OUR INDUSTRY EXPERTISE

Partnering with FUJIFILM Irvine Scientific enables your cytogenetics laboratory to leverage 50 years of experience and expertise in cell culture, and delivers the personal attention and expert level of industry knowledge you require. Our team is fully engaged, responsive to your needs, and passionate about your success.

We are by your side for any questions or concerns you may have related to FUJIFILM Irvine Scientific products.

We provide information as needed including:

- Product storage, usage, shelf life and/or stability
- Culture conditions and protocols
- Product specifications, performance, functions, and recommendations
- IFU and documentation requests

Discover our expertise. Learn more about FUJIFILM Irvine Scientific cytogenetics products today—and when you're ready, don't hesitate to contact our cytogenetics experts.
www.irvinesci.com/contact-us

www.irvinesci.com

FUJIFILM
Value from Innovation

FUJIFILM IRVINE SCIENTIFIC – CORPORATE
1830 E Warner Avenue, Santa Ana, CA 92705 USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
Support: fisitmrequest@fujifilm.com

FUJIFILM Irvine Scientific, its logo, CHANG, and CHANG Amnio are registered trademarks of FUJIFILM Irvine Scientific, Inc. in various jurisdictions.

© 2020 FUJIFILM Irvine Scientific P/N 009040 Rev.00