FUJIFILM
Value from Innovation

IrvineScientific

CHANG Amnio Recommended Protocol for CVS

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.

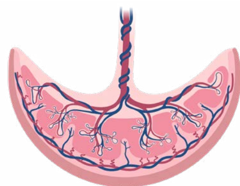
CHANG Amnio complete medium contains gentamicin sulfate (50 mg/L). Additional antibiotics may be added if desired.

DIRECTIONS FOR USE

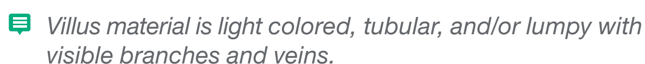
Chorionic Villi Sample Preparation:

1. Label a Petri dish for each specimen received, and transfer contents of sample aseptically. Add 5 mL of the prewarmed CHANG Amnio complete medium to the dish, and place in an incubator for 30 minutes, at 35–39°C, 5–8% CO_2 atmosphere, to allow the blood to settle.
2. Clean specimen using a dissecting microscope, initially under 1.5X magnification, then adjusting to about 3X magnification.

   Approx. 20–40 mg of chorionic villi sample is required.

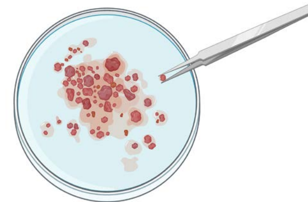


3. Using two pairs of sterile forceps, remove blood clots and any maternal decidua from the villus material, while working within the dish, under the dissecting microscope.

   
   Villus material is light colored, tubular, and/or lumpy with visible branches and veins.

4. Transfer clean villi to another Petri dish containing prewarmed CHANG Amnio complete medium. Perform final cleaning, using forceps to grasp villi and agitate gently.

   5 mg is the ideal amount to use per culture.
   Be careful to avoid damaging the fragile villi.



5. Transfer villi and media to a 15 mL centrifuge tube, and add 4 drops of antibiotic (i.e. gentamicin sulfate, 50 µl/mL) to the centrifuge tube. Let sit for 30 minutes.
6. Centrifuge villi at approx. 1,400 rpm for 5 minutes.

Chorionic Villi Sample Culture:

1. Aspirate supernatant from the centrifuged tube, making sure to leave 0.5 mL of media above cell pellet (or about 2X volume of pellet).
2. Gently resuspend pellet. Add 2 mL of prewarmed CHANG Amnio complete medium to centrifuge tube.
3. Add 2 mL of Trypsin EDTA and incubate culture undisturbed at 35–39°C, 5%–8% CO_2 atmosphere for 10 minutes.
4. Remove tube from incubator, resuspend pellet, and place in incubator for 10 additional minutes.

5. Remove centrifuge tube from incubator, resuspend pellet, and centrifuge for 8–10 minutes at 1,400 rpm.
6. Aspirate supernatant from the centrifuged tube. Resuspend pellet, then add 1 mL of collagenase to tube and place in incubator for 5 minutes.

CHANG Amnio Recommended Protocol for CVS

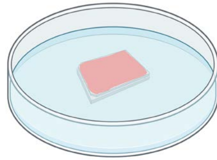
Continuation:

7. Remove from incubator and visually check to see if pellet is cloudy and no distinct pieces of individual villi can be seen. If pellet is not cloudy, place back in incubator for 5 more minutes.

8. Add 3 mL of prewarmed CHANG Amnio complete medium to centrifuge tube to stop the action of the collagenase.

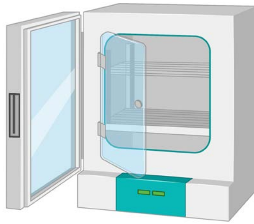
9. Centrifuge tube for 8–10 minutes at 1,400 rpm.

10. Aspirate supernatant from the centrifuged tube, leaving 0.5 mL of media above cell pellet. Resuspend pellet before adding CHANG Amnio complete medium used for set-up.



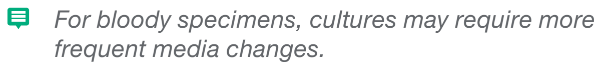
11. Set-up optimal number of cultures using 0.5 mL of CHANG Amnio complete medium per culture for each Petri dish that contains a coverslip.

12. Incubate cultures undisturbed at 35–39°C, 5%–8% CO_2 atmosphere.



13. Flood cultures on day 2 by adding 1.5 mL of prewarmed CHANG Amnio complete medium.

14. At 4 days, cultures should be checked for growth. If growth is observed, remove media and add 2 mL of fresh prewarmed CHANG Amnio complete medium to each coverslip. Cultures should be fed every 2 days thereafter.



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

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

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.

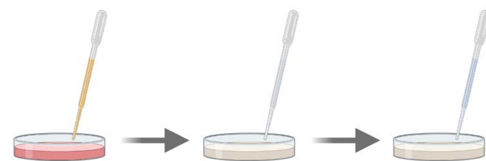
3. Slowly add 2 mL of prewarmed (35–37°C) sodium citrate hypotonic solution to the first dish, with a Pasteur pipette, being careful not to disturb the coverslip.

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

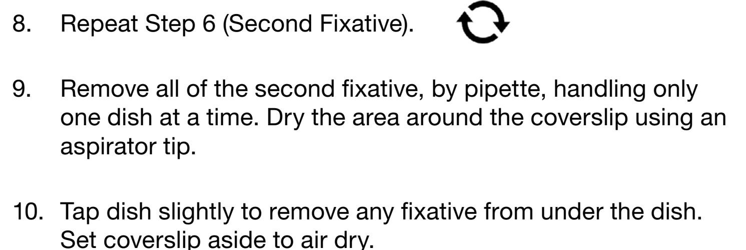
5. Add dropwise, 1 mL of fresh Carnoy's Fixative (3:1 Methanol: Glacial Acetic Acid) working with one dish at a time. Leave for 15 minutes.

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).



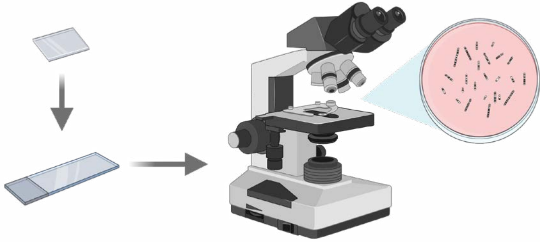
9. Remove all of the second fixative, by pipette, handling only one dish at a time. Dry the area around the coverslip using an aspirator tip.

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

CHANG Amnio Recommended Protocol for CVS

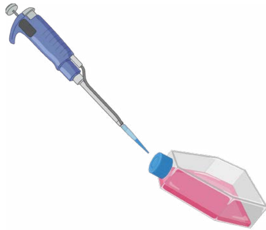
Continuation:

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.



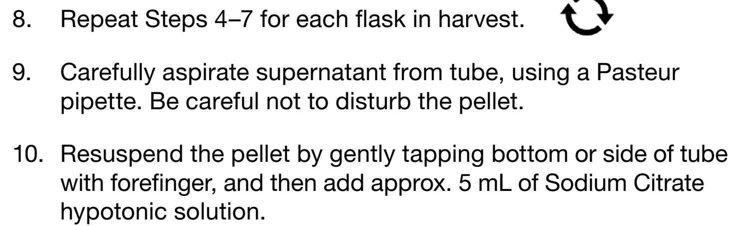
***Harvesting Flasks*:**

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.

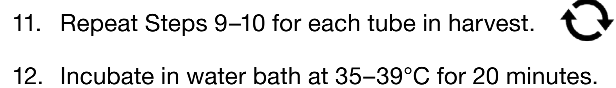


4. Add approx. 2 mL of Trypsin EDTA solution to the flask, and incubate for approx. 3 to 4 minutes.
5. Remove flask, after incubation period, and observe under inverted microscope to see if the cells are floating freely and no longer attached to the bottom of the flask.
6. Transfer contents of flask 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.

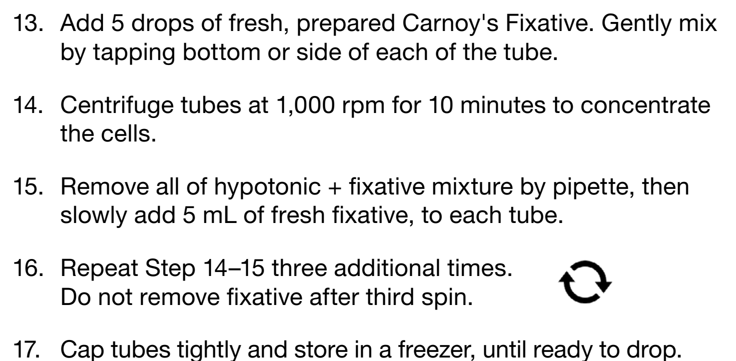
8. Repeat Steps 4–7 for each flask in harvest.
9. Carefully aspirate supernatant from tube, using a Pasteur pipette. Be careful not to disturb the pellet.
10. Resuspend the pellet by gently tapping bottom or side of tube with forefinger, and then add approx. 5 mL of Sodium Citrate hypotonic solution.



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 tube.
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 CVS

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 001206 Rev.01