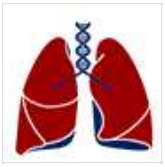

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Thawing MRC5 Sub-Stock

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1 *Works for me* This protocol is published without a DOI.

Chelseahortman

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ABSTRACT

MRC5 Media
450uls DMFM/F12 (Cell Center cat#11320033)
45uls 10% FBS (Invitrogen cat#10437-028)
5uls 1% pen/strep (Cell Center cat#15240096)

PROTOCOL CITATION

Morrisey Lab 2021. Thawing MRC5 Sub-Stock. **protocols.io**
https://protocols.io/view/thawing-mrc5-sub-stock-bs5kng4w

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1 Set aside 10mL of cold MRC5 media in 15mL conical tube.

2 Take 1 MRC5 sub stock aliquot out of liquid nitrogen.

3 Warm aliquot in

4 bath

5 Note** It is important to thaw until last ice crystal and immediately add to MRC5 media

6 From the conical tube with 10mL of cold MRC5, using a P1000, add 200uls of MRC5 media to the sub stock aliquot, slowly mix and then add the entire aliquot to 10mL of MRC5 media.

7 Mix gently with 10mL serological pipette on slow settings.

8 Spin at 250g(rcf) for 10 min.

9 Discard supernatant and wash pellet with 1mL cold MRC5 media.

10 Add an additional 10mL cold MRC5 media and spin at 250g for 10 min.

11 10. Label T75 flask with your initials, date, and Px+1(passage).

12 11. Resuspend pellet in 10mL of cold MRC5 media and add to T75 flask.

13 12. Make sure to distribute the cells evenly by gently moving the flask up and down, side to side, and put in appropriate incubator.

14 13. Check on media and fibroblasts every 1-2 days, making sure they are growing.

15 14. Split fibroblasts in approximately 5 days.

16 15. Make sure to not overgrow and keep until passage 10.