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Thawing of CT-2A cell line

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protocol .

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This protocol describes the thawing of mouse CT-2A glioma cells

Guido Krähenbühl 2022. Thawing of CT-2A cell line. **protocols.io** https://protocols.io/view/thawing-of-ct-2a-cell-line-b8gurtww

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GMO safety level 1

DMEM **Thermo Fisher Scientific Catalog #41966**

FBS **Invitrogen - Thermo Fisher**

Make sure to warm up media to **37 °C**

1 Remove cryovial from liquid nitrogen storage and place on dry-ice for transfer to cell culture lab.

Cryovial IDs	
Additional Identifiers	

2 Remove cryovial from dry-ice and hold in a **37 °C** waterbath until thawed

Avoid submerging the lid or rim of the cryovial in the waterbath as this may result in contamination

Dry the cryovial thoroughly, spray with 70% ethanol and transfer to cell culture hood

3 Transfer contents of cryovial to a Falcon 15 mL Polystyrene Conical Tube **Fisher Scientific Catalog #352095**

containing **7 mL DMEM, 10% FBS, no antibiotics** .

3.1 Rinse the cryovial with **1 mL** of the cell suspension and add it back to the same Falcon 15 mL Polystyrene Conical Tube **Fisher Scientific Catalog #352095**

4 5m

Centrifuge at **300 rcf, Room temperature, 00:05:00**

5 Discard supernatant, resubstitute with fresh **7 mL DMEM, 10% FBS, no antibiotics**

6 Transfer cell suspension to a

Petri dish, 10cm, polystyrene **Fisher Scientific Catalog #FB0875712** and agitate gently back and forth and side to side to evenly distribute cells

7 Place the petri dish in a **37 °C Incubator** (5%CO_2)

8 After 24 hours, inspect adherence and confluency.

If a major amount of cells did not attach, remove the media and replace with **7 mL DMEM, 10% FBS, no antibiotics** and check for surviving cells after 24 hours