

Giardia supernatant cleansing protocol

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Abstract

This protocol provides an inexpensive and fast way to cleanse the supernatant of *Giardia* trophozoites' culture from bovine proteins and any other protein digests present in TYI-S-33.

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Before start

Warm non-supplemented DMD+Pen/Strep (1%) and 1xPBS to 37°C.

Protocol

Detach parasites from tubes' walls

Step 1.

Put culture tubes on ice for 20 min then manually and slowly shake up and down tubes 15 times or more.

Transfer samples

Step 2.

Transfer samples into 15ml Falcon plastic tubes.

SAFETY INFORMATION

Transfer has to be done in a Biosafety cabinet, under sterile conditions.

Pellet down parasites

Step 3.

Centrifuge samples at 3,000 rpm for 10 min, at room temperature.

Trophozoites wash #1

Step 4.

Remove supernatants and resuspend pellets in 4 ml 1xPBS and repeat centrifugation (3,000 rpm for 10 min, at room temperature).

AMOUNT

4 ml Additional info: 1x PBS

SAFETY INFORMATION

All following steps are performed in Biosafety cabinet in sterile environment.

Trophozoites wash #2

Step 5.

Remove supernatants and resuspend pellets in 2 ml 1xPBS and repeat centrifugation.

AMOUNT

2 ml Additional info: 1xPBS

Trophozoites wash #3

Step 6.

Remove supernatants and resuspend pellets in 1 ml 1xPBS and repeat centrifugation.

AMOUNT

1 ml Additional info: 1xPBS

Supernatant cleansing

Step 7.

Remove supernatants, resuspend pellets in 1 ml of non-supplemented DMEM+ Pen/Strep (1%), transfer samples in glass tubes with filter caps and incubate for one hour at 37°C, +5% CO₂.

AMOUNT

1 ml Additional info: non-supplemented DMEM+ Pen/Strep (1%)

TEMPERATURE

37 °C Additional info: Incubation at +5% CO₂

NOTES

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Incubation for longer than an hour causes parasites to die.

Detach trophozoites from tubes' walls

Step 8.

Put samples on ice for 5 min and slowly shake them to detach parasites from the walls.

Pellet down trophozoites

Step 9.

Transfer samples in 15 ml Falcon tubes and centrifuge (3,000 rpm for 10 min, at room temperature).

Collect supernatant

Step 10.

Collect supernatants in labeled 1.5 ml Eppendorf tubes and store both pellets and supernatants samples at -20°C.

TEMPERATURE

-20 °C Additional info: Storage

Warnings

Giardia is infectious to humans, work under a Biosafety cabinet level 2 or up following the laboratory health and safety regulations.