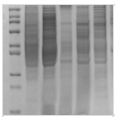

Mar 04, 2020

# Protein extraction form *Aurantiochytrium limacinum* (ATCC MYA-1381)

Anbarasu Karthikaichamy[1]

[1]State University of New York at Stony Brook

1 Works for me dx.doi.org/10.17504/protocols.io.bc7gizjw

Collier Lab

Anbarasu Karthikaichamy
State University of New York at Stony Brook

ABSTRACT

Protein extraction protocol for *Aurantiochytrium limacinum* (ATCC MYA-1381; Stramenopile/ Heterokont, Thraustochytrid).

SAFETY WARNINGS

The lysis buffer contains detergents, and therefore it should be handled with care. Always wear gloves to minimize keratin contamination.

BEFORE STARTING

Get a bucket full of ice, and always keep the lysed culture or protein suspension on ice.

## Lysis buffer preparation 10m

1 Start by making stocks of the following, 10m

- [M]**1 Molarity (M)** KCl
- [M]**25 Milimolar (mM)** $MgCl_2$
- [M]**1 Molarity (M)** Tris

Prepare lysis buffer (LyB) by adding the following chemicals (for 10ml),

| Chemical | Volume (uL) |
|---|---|
| KCl (1M) | 500 |
| MgCl2 (25mM) | 1000 |
| Tris (1M) | 500 |
| NP40 (100%) | 45 |
| Tween (100%) | 45 |
| dH2O | 8310 |



This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## Cell harvesting 10m

2 10m

- Pipette out ~3 ml of cell suspension from the test tube or conica flask.
- Centrifuge the cells at **3000 rpm, 4°C 00:10:00**
- Discard the supernatant and keep the cell pellet on ice.

## Lysis and soluble fraction extraction 20m

3 20m

- Add 1 ml of lysis buffe (LyB) to the pellet.
- Vortex the mixture for 20 mins on high spped.
- Centrifuge the suspension at **13500 rpm, 4°C 00:15:00**
- Collect the supernatant in a separate tube and place it on ice. This is the soluble fraction.

## Insoluble fraction extraction 40m

4 40m

- Resuspend the pellet from the above step in 4x LDS sample buffer
- Add required amount of $dH_2O$
- Add 1:10(v:v) 25% β-Mercaptoethanol

Always handle β-Mercaptoethanol in fume hood!

- Heat the mixture at **70 °C** for **00:10:00**
- Centrifuge the mixture at **13500 rpm, 4°C 00:20:00**
- Carefully aspirate the supernatant (insoluble fraction) into a fresh tube and keep it on ice.

Alternatively, one could also use custom buffers (with high concentration of detergent) for insoluble fraction extraction.

## Quantification and visualization

5 Quantify the protein extract using an appropriate mehtod and the proteins can be visualized on a SDS-PAGE gel stained with coomassie blue.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited