# Cell lysis, detergent-free

**Queenie Chan**

## Abstract

Detergents are generally not compatible with mass spectrometers, so this is a detergent-free method of cell lysis that is compatible with mass spectrometry. Since this protocol does not have a precipitation step, it saves time and minimizes sample loss as well.

**Citation:** Queenie Chan Cell lysis, detergent-free. **protocols.io**
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## Guidelines

Mix well after each step by vortex for 2 seconds.

## Before start

Prepare ice bucket.

Prepare 90°C heat block.

## Protocol

Cell sample

**Step 1.**

Fresh or previously frozen, store on ice. Original protocol uses $5x10^7$ cells per sample.

Resuspend cells

**Step 2.**

AMOUNT

1.5 ml Additional info: ice cold Milli-Q water

Add trifluoroethanol (TFE)

**Step 3.**

AMOUNT
1.5 ml Additional info: Trifluoroethanol
SAFETY INFORMATION
**This step should be done in the fume hood.**

NOTES
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1:1 water-TFE acts as a hypotonic aqueous buffer to lyse cells, eliminating the need for detergent. TFE helps protein solubility and denaturation; it readily evaporates, so removing it is easy.

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TFE is located in NCE 435 - flammable cabinet

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TFE evaporates fast, so work quickly.

Sit on ice
**Step 4.**

Vortex
**Step 5.**

Sonicate
**Step 6.**

NOTES
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Original protcol calls for 2 mins at 20% duty cycle, output control 3 using a ultrasonic probe. The time required to do this for large number of samples is not realistic, and therefore not used here.

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While this step is happening, turn on the heat block and set it at 95oC

Adjust pH
**Step 7.**

AMOUNT
200 µl Additional info: Tris pH 8.5, 100mM

Remove debris
**Step 8.**

Spindown debris at top speed on a benchtop centrifuge for 10 minutes, then keep the supernatant.

## Measure protein concentration

**Step 9.**

Use the Coomassie reagent to measure protein concentration. Aliquot the desired amount to proceed with the remainder of this experiment.

NOTES

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Freezing unused protein is not recommended since there is a high chance of precipitation upon thawing.

## Reducing agent

**Step 10.**

Add TCEP (tris(2-carboxyethyl)phosphine to a final concentration of 10mM

NOTES

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TCEP is located at NCE 436 -20C freezer door

## Alkylating agent

**Step 11.**

Add 2-chloroacetamide to a final concentration of 40mM

NOTES

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2-chloroacetamide is located in NCE 438 - chemical shelves

## Preparing the lysate for digestion

**Step 12.**

AMOUNT

15 ml Additional info: 50mM ammonium bicarbonate

## Digestion with LysC

**Step 13.**

Add 1ug LysC to every 50ug protein

TEMPERATURE

37 °C Additional info: incubator (not heat block)

## Digestion with trypsin

**Step 14.**

Add 1ug trypsin to every 50 ug protein. Allow reaction to occur overnight.

TEMPERATURE

37 °C Additional info: incubator (not heat block)

STAGE tip

**Step 15.**

Proceed with desalting as normal