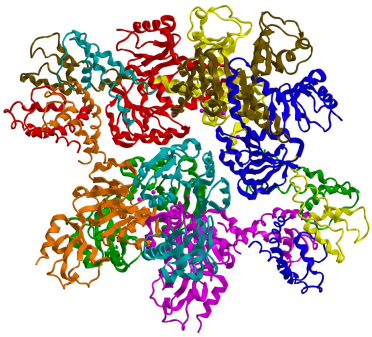
Chromatrap

# Chromatrap® Protein Extraction Kit

Buffers for extracting total,
cytoplasmic and nuclear protein

*Product no. 500283*



# Contents

# Kit Components

| Component | Volume | Storage Temperature |
|---|---|---|
| 10 x Cytoplasmic Buffer | 20ml | 4°C |
| Nuclear Buffer | 20ml | 4°C |

*Table 1: Kit components and storage temperatures*

Reagents must be stored as directed on receipt, if stored correctly reagents are stable for up to 1 year.

## Additional materials and reagents required

- Cell scrapers
- 1.5mL microcentrifuge tubes
- Vortex microcentrifuge (4°C)
- Phosphate buffered saline (PBS)
- Protease inhibitors (PIC)
- 1M Dithiothreitol (DTT)
- Phosphatase Inhibitors (Optional)

# Introduction

The Chromatrap® Protein Extraction kit is a fast and efficient method of extracting cytoplasmic, nuclear or total protein from mammalian cells. The optimised reagents and protocol provide a high yield of protein with low cross-fraction contamination in under 1.5 hours.

Proteins isolated using the Chromatrap® Protein Extraction kit can be used in a wide array of downstream techniques such as protein immunoprecipitation, PAGE and western blotting, ELISA, EMSA and reporter assays. The Cytoplasmic Buffer does not contain ionic detergents allowing proteins to be extracted in their native state and is suitable for downstream activity assays.

Sufficient reagents are provided to perform 100 cytoplasmic and nuclear or total protein extractions. From 8 million cells, the kit typically yields 1000 µg cytoplasmic and nuclear protein and 2000 µg total protein

Specific extraction of nuclear proteins from cells is central to the success of many gene regulation studies. A variety of methods exist to isolate nuclei and prepare nuclear protein extracts, most of these are lengthy processes requiring mechanical homogenization, manual handling, freeze/thaw cycles, extensive centrifugation or dialysis steps that may compromise the integrity of fragile nuclear proteins. The Chromatrap® Protein Extraction Kit overcomes these problems, providing high-quality, concentrated protein extracts for use in downstream analysis techniques.

| Extraction | Time (minutes) |
|---|---|
| Cytoplasmic Protein | 45 |
| Nuclear Protein | 90 |
| Total Protein | 60 |

*Table 2: Protein extraction assay times*

## Features of the Chromatrap® Protein Extraction Kit

- **Fast** - Obtain non-denatured, active proteins purified in 90 minutes.
- **Simple** - No ultracentrifugation over gradients.
- **Efficient** - Minimum cross-contamination amongst fractions.
- **Compatible** - Extracted proteins can be used in many downstream assays, including Western blotting, gel-shift assays, protein assays, reporter gene assays and enzyme activity assays.

# Preparation of Reagents

**Cytoplasmic Buffer** is supplied as a 10x solution and must be diluted to a 1x final concentration with sterile distilled water **before use**. Once diluted, do not store 1x Cytoplasmic Buffer for more than 1 week.

PIC and DTT (1mM final concentration) must be added to **Cytoplasmic and Nuclear Buffers immediately before use**. Do not store Cytoplasmic and Nuclear Extraction solutions once PIC and DTT have been added.

Prepare sufficient extraction solutions from the supplied buffer solutions for the number of extractions to be performed according to Table 3 below. The volumes in Table 3 are for one extraction from a single plate or culture flask ($75cm^3$).

| Component | Cytoplasmic Extraction Buffer | Nuclear Extraction Buffer | Total Extraction Buffer |
|---|---|---|---|
| **10 x Cytoplasmic Buffer** | 100µl | - | - |
| **Nuclear Buffer** | - | 100µl | 500µl |
| **Distilled Water** | 890µl | - | - |
| **PIC*** | 10µl | 1µl | 5µl |
| **DTT** | 1µl | 0.1µl | 0.5µl |
| **Total** | 1ml | 100µl | 500µl |

*Table 3: Buffer volumes for extraction buffer preparation*

* The composition and quantity of protease inhibitor will vary depending on the cell type. This may require optimisation by the user. Volumes indicated are guidelines.

# Cytoplasmic and nuclear extraction protocol

## Step 1: Cytoplasmic Protein Extraction

*Adherent cells*

1. Decant media from flask and wash cells twice in an appropriate volume of ice-cold PBS.
2. Detach cells by scraping in 1mL ice cold PBS with a cell scraper and transfer suspension to a 1.5mL microcentrifuge tube by pipetting.
3. Collect cells by centrifugation 5000 x g for 5 min, 4°C. Discard supernatant.
4. Gently re-suspend cells in 1mL **pre-prepared Cytoplasmic Extraction Buffer**. If required, add appropriate phosphatase inhibitors.
5. Incubate on ice for 15 min to lyse cell membranes.
6. Vortex suspension for 10 s on high setting.
7. Pellet nuclei and cell debris by centrifugation at 5000 x g, 10 min at 4°C.
8. Transfer supernatant containing cytoplasmic fraction to a clean dry 1.5mL microcentrifuge tube and retain pellet for nuclear extraction. Store cytoplasmic protein fraction at -80°C until use.

*Suspension cells*

1. Pellet cells by centrifugation 5 min, at 200 x g at 4°C.Discard supernatant and wash cells twice in an appropriate volume of ice-cold PBS.
2. Re-suspend cells gently in 1mL **pre-prepared Cytoplasmic Extraction Buffer**. If required, add appropriate phosphatase inhibitors.
3. Incubate on ice for 15 min to lyse cell membranes.
4. Vortex suspension 10 s on high setting.
5. Pellet nuclei and cell debris by centrifugation at 5000 x g, 10 min at 4°C.
6. Transfer supernatant containing cytoplasmic fraction to a clean dry 1.5mL microcentrifuge tube and retain pellet for nuclear extraction. Store cytoplasmic fraction at -80°C until use.

## Step 2: Nuclear Protein Extraction

1. Re-suspend nuclear pellet in 100µl **pre-prepared Nuclear Extraction Buffer** by vortexing on the high setting.
2. Incubate the suspension on ice for 30 min, vortexing on the high setting for 30s at 5min intervals.
3. Pellet cell debris by centrifugation 10 min at 14,000 x g at 4°C.
4. Transfer supernatant containing nuclear fraction to a clean dry microcentrifuge tube and store at -80°C until use.

# Total protein extraction protocol

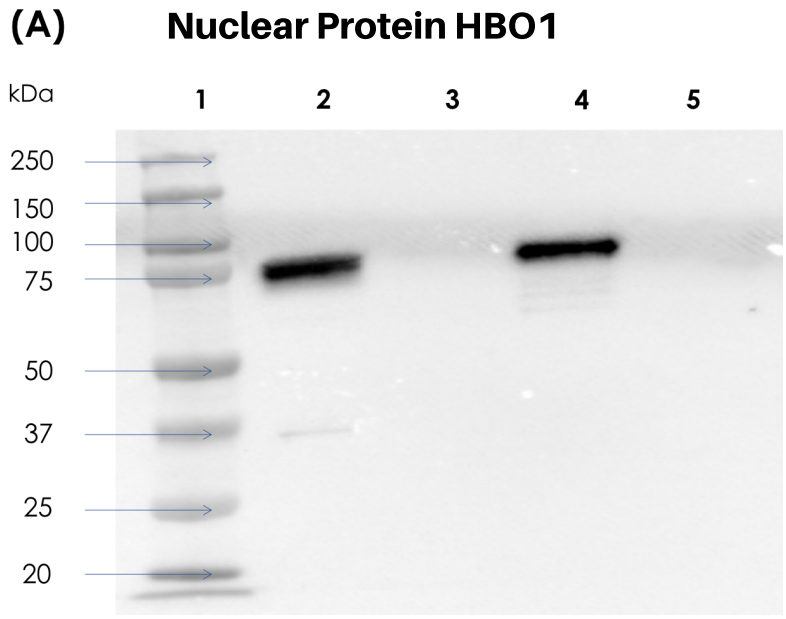
*Adherent cells*

1. Decant media from flask and wash cells twice in an appropriate volume of ice-cold PBS.
2. Detach cells by scraping in 1mL ice cold PBS with a cell scraper and transfer suspension to a 1.5mL microcentrifuge tube by pipetting.
3. Collect cells 5000 x g, 5 min, 4°C. Discard supernatant.
4. Re-suspend pellet in 500µl **pre-prepared Total Extraction Buffer** by vortexing on the high setting.
5. Incubate the suspension on ice for 30 min, vortexing on the high setting for 30 s at 5 min intervals.
6. Pellet cell debris by centrifugation, 10min at 14,000 x g at 4°C.
7. Transfer supernatant containing total protein to a clean dry microcentrifuge tube and store at -80°C until use.

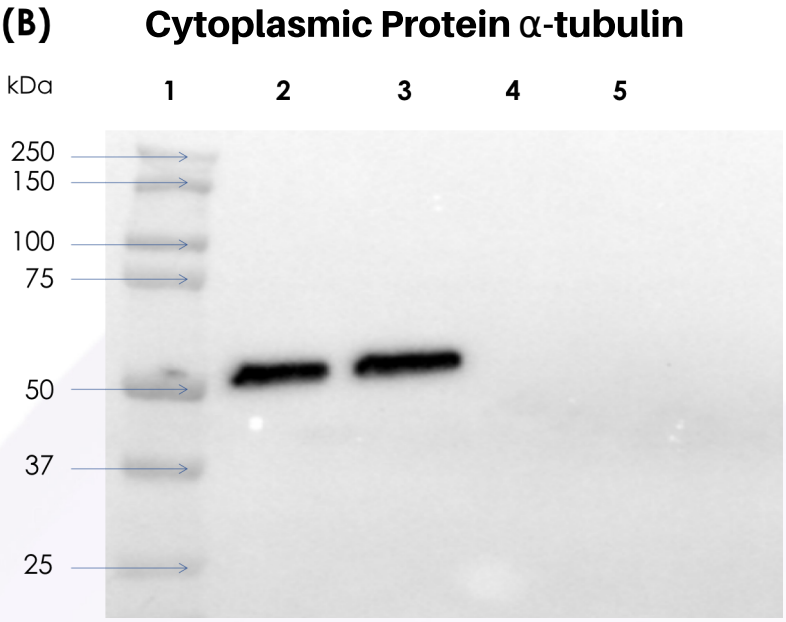
*Suspension cells*

1. Pellet cells by centrifugation 5 min, at 200 x g at 4°C. Discard supernatant and wash cells twice in an appropriate volume of ice-cold PBS.
2. Re-suspend pellet in 500µl pre-prepared Total Extraction Buffer by vortexing on the high setting.
3. Incubate the suspension on ice for 30 min, vortexing on the high setting for 30s at 5min intervals.
4. Pellet cell debris by centrifugation 10min at 14,000 x g at 4°C.
5. Transfer supernatant containing total protein to a clean dry microcentrifuge tube and store at -80°C until use.

# Appendix




1. Dual colour protein standard
2. Total protein extraction
3. Cytoplasmic fraction
4. Nuclear Fraction
5. Pellet




1. Dual colour protein standard
2. Total protein extraction
3. Cytoplasmic fraction
4. Nuclear Fraction
5. Pellet

Figure 1: Western blot of (A) the nuclear protein HBO1 and (B) the cytoplasmic protein α-tubulin of protein extracts from total, cytoplasmic and nuclear fractions extracted with the Chromatrap Protein Extraction Kit.

# Troubleshooting and FAQ's

### Why do I have a low concentration of protein in my cytoplasmic fraction?

Ensure cells have been efficiently lysed during step 5 of the adherent cell or step 3 of the suspension cell protocol. Vortex cells briefly after 15 min incubation on ice. If necessary, check cells under a phase contrast microscope after lysis.

### Why do I have a low concentration of protein in my nuclear fraction?

Ensure cells have been efficiently lysed during step 5 of the adherent cell or step 3 of the suspension cell protocol. Vortex cells briefly after 15 min incubation on ice. If necessary, check cells under a phase contrast microscope after lysis.

### Why do I have poor fractionation of cytoplasmic and nuclear proteins?

Ensure the cytoplasmic fraction is fully aspirated from above the pellet prior to nuclear lysis, residual liquid on top of the pellet will contain cytoplasmic proteins. Incomplete lysis of the cell membrane during lysis of the cytoplasmic fraction can also lead to carryover of cytoplasmic proteins in the nuclear fraction. If nuclear proteins are present in the cytoplasmic fraction the incubation time with Cytoplasmic Buffer may need to be reduced. Frozen cells will be more sensitive to lysis and incubation times should be reduced accordingly.

### Why do I have a low concentration of protein in my nuclear fraction?

Ensure cells have been efficiently lysed during step 5 of the adherent cell or step 3 of the suspension cell protocol. Vortex cells briefly after 15 min incubation on ice. If necessary, check cells under a phase contrast microscope after lysis.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