

# EZExtract™ Polyubiquitin Buffer Kit

Rev 11/14

Store the Extraction buffer at 4°C and the Protease Inhibitors and NEM at -20°C.

Cat. No.: K6570-3

Contains: Extraction Buffer, Protease Inhibitors and NEM

## Description:

BioVision's EZExtract™ Polyubiquitin Buffer Kit is ideal for efficient extraction of polyubiquitin modified proteins from cells. Ubiquitin is a highly conserved 76-amino acid protein. It can be conjugated via its C-terminus to the amine groups of lysine residues on target proteins. This conjugation is referred to as monoubiquitylation. Additional ubiquitin moieties can be subsequently conjugated to this initial ubiquitin, utilizing any one of the seven lysine residues on the surface of ubiquitin. The formation of these ubiquitin chains is referred to as polyubiquitylation. Different types of polyubiquitin chains can form, depending on the internal lysine residue used for this conjugation. These polyubiquitin chains further can attach to proteins post-translationally and aid in numerous downstream activities like proteasome-mediated proteolysis, autophagy, DNA damage tolerance, inflammation, apoptosis, signal transduction etc. Several classes of Ubiquitin interacting proteins help in mediating these downstream effects. BioVision's EZExtract™ Polyubiquitin Buffer kit efficiently extracts polyubiquitinated proteins from cells while preserving the ubiquitination state of protein samples.

## Key Features:

- Highly efficient in lysing cells.
- Preserves ubiquitination or sumoylation status during downstream processing.
- Compatible with mass spectrometry.

## Applications:

- Extraction of cells/tissues to achieve optimal yield of ubiquitinated or sumoylated proteins.
- General lysis buffer for a broad range of applications, including Western blot, ELISA, dot blot, enzymatic assays, etc.

## Kit Contents:

Components	Quantity	Cap Color Code	Part Number
Extraction Buffer	30 ml	NM	K6570-3-1
Protease inhibitors	1 vial	Red	K6570-3-2
NEM solution	1 ml	Blue	K6570-3-3

## Storage and Handling:

Upon receiving the kit, store **Extraction Buffer at 4°C** and the **Protease inhibitors and NEM solution at -20°C**.

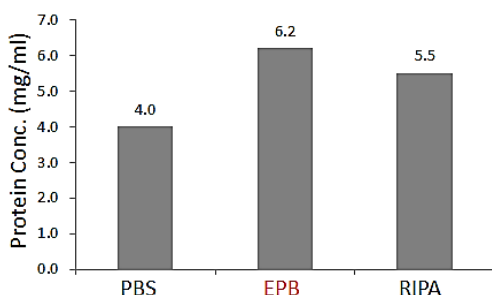
## Buffer Preparation:

- Before the first use, reconstitute the Protease inhibitors with 250 µl DMSO, pipette to dissolve all powder. Aliquot and store at -20°C for later use. Prevent repeated freeze thaw..
- Add 10 µl Protease inhibitors, 30 µl NEM solution to 1 ml Extraction Buffer, scale up accordingly if more buffer is needed, mix well, the EZExtract™ Polyubiquitin Buffer is ready for use.

## Protein Extraction Protocol:

1. Prepare sufficient volume of the Extraction Buffer as described above and keep on ice.
2. Collect suspension cells by centrifugation at 500 x g for 5 minutes at 4°C. Collect adherent cells by trypsinization or using a cell scraper, before the centrifugation.
2. Re-suspend cells in 1X ice-cold PBS, centrifuge again at 500 x g for 5 minutes at 4°C. Discard the supernatant and keep the cell pellet.
3. Add 5-15 volumes (V/W) of EZExtract™ Polyubiquitin Buffer and vortex at maximum speed for 5 seconds.  
 Note: For tissue samples, homogenize tissues in 3-4 volume (W/V) of the Extraction Buffer.
3. Incubate the samples on ice for 10 minutes, with intermittent mixing.
4. Centrifuge the samples in a microcentrifuge at 15,000 X g at 4°C for 15 minutes.
5. Collect the supernatant.

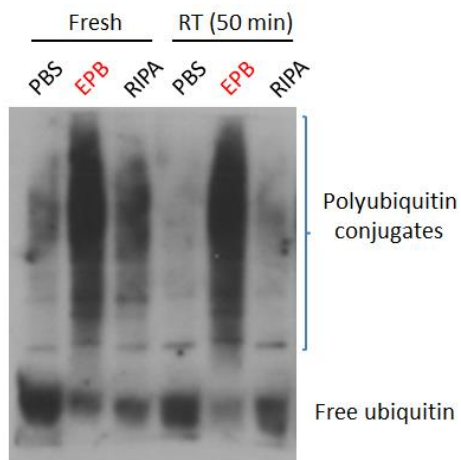
Figure 1



**Figure 1. Protein extraction with EZExtract™ Polyubiquitin Buffer kit.**

Jurkat cell pellet were lysed with BioVision complete EZExtract™ Polyubiquitin Buffer (EPB), or RIPA buffer with protease inhibitors, or sonicated in PBS with protease inhibitors (the same ratio of volume to cell pellet weight used across board). Protein concentration was determined with Bradford method (BSA as standard). BioVision's EZExtract™ Polyubiquitin Buffer extracts more proteins than RIPA buffer or sonication (PBS).

Figure 2



### Figure 2. Extraction of cellular polyubiquitinated proteins with EZExtract™ Polyubiquitin Buffer kit.

Jurkat cells were lysed with BioVision's EZExtract™ Polyubiquitin Buffer (EPB), or RIPA buffer with protease inhibitors (RIPA), or homogenized by sonicating in PBS with protease inhibitors (PBS). 30 µg each of 3 different cell lysates were boiled in 1 X SDS-PAGE loading buffer immediately after preparation (Fresh). Another set of the same 3 samples were incubated at Room Temperature (RT) for 50 min (RT(50 min)) before boiling in the 1 x SDS-PAGE buffer.

Both sets of sample (FRESH or RT(50 min)) were separated in a 4-20% gradient gel, and transferred onto PVDF membrane for Western blot analysis using ubiquitin antibody.

EZ Extract™ Polyubiquitin Buffer (EPB) kit yields notably higher amount of poly-ubiquitinated proteins than either sonication or RIPA buffer, as observed in the Fresh samples

EZ Extract™ Polyubiquitin Buffer (EPB) prevents the polyubiquitinated proteins from de-ubiquitination during their extended incubation at room temperature (RT 50 Min).

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 Hi-Bind™ Ni QR Agarose Beads (6562)  
 Benzonase Nuclease (Cat. #7680)  
 10K Spin Column (1997)  
 Ready-to-use Ni QR Agarose Beads Buffer Kit (K6563-3)  
 Protein G-Sepharose Column (6518)  
 Protein A/G-Sepharose Column (6528)

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**FOR RESEARCH USE ONLY! Not to be used on humans.**