

## **A Rapid Method for Purification of Ubiquitinated Proteins**

### **Background**

The ubiquitin/proteasome system is the major intracellular non-lysosomal proteolytic system that catalyzes the degradation of many regulatory proteins. Ubiquitin is a highly conserved 76 amino acid residue protein present in all eukaryotes. Conjugation of ubiquitin molecules to proteins marks them for degradation via the ubiquitin/proteasome pathway. Critical regulators of cell growth, differentiation, apoptosis and tumor suppression are targets of this proteolytic pathway, and include p53,  $\beta$ -catenin, c-Jun, c-Myc, I $\kappa$ B $\alpha$  and Bcl2.

The presence of ubiquitinated proteins is often one of the hallmarks of pathological conditions such as neurodegenerative diseases and cancer. Consequently, antibodies to ubiquitin, ubiquitin activating and conjugating enzymes, ubiquitin protein ligases, and ubiquitin deconjugating enzymes have shown these proteins to be associated with pathogenesis. The use of anti-ubiquitin antibodies for purification of ubiquitinated proteins has the following disadvantages:

- i. Anti-ubiquitin antibodies preferentially recognize denatured form of ubiquitin. Consequently, the use of antibodies to purify ubiquitinated proteins involves harsh denaturation steps.
- ii. The preparation of monoclonals is time consuming and costly

**The present invention describes the first definitive method to selectively isolate ubiquitinated proteins involved in critical cellular events.**

### **Description of the Technology**

A protein critical to eukaryotic DNA excision repair mechanism was shown to bind ubiquitinated proteins. Specifically, a domain in this protein was shown to bind ubiquitinated cellular proteins with high affinity. This binding affinity was exploited to prepare an affinity matrix by coupling the ubiquitin-binding protein directly to Sepharose beads. This affinity matrix represents a very efficient tool to selectively purify ubiquitinated proteins from any target sample in amounts sufficient for further analyses. A Kit for the purification and recovery of ubiquitinated proteins has been developed.

### **Advantages**

- The method is simple (one-step), gentle (physiological buffers) reproducible and efficient.
- The purification and recovery steps are also rapid, and can be completed in less than 1 hour.
- The purification protocol requires less than 0.1 mg of protein.
- Sample preparation time is minimal
- The interaction between the ubiquitin-binding domain and ubiquitinated substrates is strong and tolerant to high salt and denaturing conditions
- This affinity matrix can be used to purify ubiquitinated proteins from any source since ubiquitin is conserved across all eukaryotic species.

### **Applications**

- . • For the isolation of low abundance cellular regulatory proteins.
- . • To test if a protein is a substrate of the ubiquitin/proteasome pathway.
- . • To generate antibodies against the purified proteins.

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### **Patent Status**

- . •PCT application filed.
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### **Licensing Opportunity**

- This technology is available for non-exclusive license.

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