

Background

In vitro ubiquitination is the ATP dependent covalent attachment of ubiquitin to a target substrate. Ubiquitin tagged proteins can, for example, be marked for destruction in the proteasome, a discovery that won the Nobel Prize in 2004. More recently, ubiquitins were found to be directed to other cellular compartments where they play important regulatory functions in a range of cellular processes, such as DNA damage responses, endocytosis and mitosis.

Abcam offers an extensive range of ubiquitins and related proteins for all aspects of ubiquitin research.

The following is a typical *in vitro* ubiquitination protocol.

Materials

The total reaction volume for this experiment is 50 μ l and this will be enough material to run approximately two lanes per gel (~ 20 μ l each).

Component	Working Concentration	Abcam product examples (please note that some product concentrations are lot-specific and will vary between different batches)
Ubiquitin protein	0.02 mg/ml	ab125831
E1	5 nM	ab125733 , typical stock concentration of 1 μ M
E2	100 nM	ab127392 , typical stock concentration of 40 μ M
E3	20 nM	Choose from a range of Abcam E3s For Parkin the typical stock concentration is 730 nM
Substrate	200 nM	lysozyme
10 x Ubiquitylation buffer	50 mM Tris8 5 mM MgCl ₂ 0.1% Tween-20 1 mM β -mercaptoethanol or DTT	
ATP stock	2 mM	
Double distilled water		

Methods

The dilutions in the protocol below are based on Abcam products.

1. Dilute E1 stock solution 1/20 in 1 x ubiquitylation buffer and add 5 μ L to 50 μ L reaction
2. Dilute E2 stock solution 1/40 in 1 x ubiquitylation buffer and add 5 μ L to 50 μ L reaction
3. Dilute ubiquitin 1/20 in 1 x ubiquitylation buffer and add 5 μ L to 50 μ L reaction
4. Add 3.5 μ L of 10 x ubiquitylation buffer
5. Add 5 μ L of 2 mM ATP solution
6. Add substrate (the amount will depend on substrate concentration)
7. Make up to 48.5 μ L with water
8. Finally add 1.5 μ L of 0.05 mg/ml E3 to 50 μ L reaction
9. Incubate the mixture at room temperature for one hour
10. Depending on protein run percentage gel
11. For visualization perform a WB using an anti-ubiquitin antibody (VU-1) ([ab128424](#)) and an antibody against the protein/substrate.

