Ubiquitinylation assays

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Abstract

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Protocol

Separation of white blood cells

Step 1.

Blood from healthy volunteers and patients was collected in PAXgene blood collection tubes (Becton Drive, Franklin Lakes, NJ, USA) and stored frozen at −80°C. Thawed samples were thawed at room temperature for 2 hours at room temperature and centrifuged at 4300 × *g* for 10 min in a swing rotor centrifuge (Kubota 5911, Tokyo, Japan; http://www.centrifuge.jp/products/model-5911/). The supernatant was discarded, sterilized water was added to the pellets, and the erythrocytes were hemolyzed. The suspension was then centrifuged at 4300 g for 10 minutes, the supernatant was discarded, and white blood cells were extracted into the resuspension solution.

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Step 2.

Human white blood cells were disrupted and ubiquitinylated proteins were recovered using a K48 Linkage Specific UbiTest kit (Life Sensors, Inc., Malvern, PA, USA); in particular, expression of K48 polyubiquitin was verified by performing taget protein immunoblot analysis on human white blood cells. As a control with the K48 deubiquitinating enzyme, ubiquitin specific peptidase 2 catalytic domain (USP2CD) was used. Ubiquitinylated proteins were resolved by SDS-PAGE.