



An accurate, high throughput method to measure site-specific ubiquitin abundance

Isotopically balanced quantification of ubiquitination (IBAQ-Ub): A rapid, sensitive method to measure site-specific ubiquitination abundance

IP Status: Pending US Patent; **Application #:** 16/688,235

A new approach to measuring protein ubiquitination

Lysine ubiquitination is an essential posttranslational modification in eukaryotic cells and is involved in a multitude of cellular processes including DNA damage and repair, subcellular localization and protein degradation. Alterations in ubiquitination of proteins are linked to many serious diseases, including cancer, neurodegenerative diseases and immunologic disorders. However, a reliable, specific, and rapid method to analyze lysine ubiquitination does not exist. A new technology developed at the University of Minnesota uses a chemical proteomics approach to specifically label ubiquitinated lysine residues in proteins using an isotopically labeled acetylglycylglycyl-NHS tag. This tag distinguishes unmodified lysine residues in the protein of interest from ubiquitinated lysine residues, and allows for quantification using mass spectrometry. Data has shown that this approach, termed isotopically balanced quantification of ubiquitination (IBAQ-Ub) is reliable, specific and has the potential to be developed into a high-throughput method to measure ubiquitination abundance.

Improving on the limitations of current methods

Compared to the current methods used to assess protein ubiquitination, IBAQ-Ub does not require the use of site specific antibodies or an extra enrichment step, saving both time and money. Furthermore, this technique does not rely on in vitro synthesis of isotopically labeled standards required by other methods (i.e. AQUA), and as such could enable global untargeted discoveries of physiologically relevant changes in protein ubiquitination. With further development, IBAQ-Ub could have clinical applications and be used to be used for biomarker studies of patient tissues.

Phase of Development

Proof of concept. Method validated using synthetic protein standards.

Key Benefits & Differentiators

- **Accurately assesses protein ubiquitination:** IBAQ-Ub method reliably measures ubiquitination stoichiometries as verified against standards. without requiring enrichment of the protein of interest, saving time and resources.
- **Saves time and resources:** Method doesn't require enrichment of the target protein, use of site specific antibodies or in vitro synthesis of labeled standards.
- **Flexible to a variety of situations:** Can be used with purified proteins or complex cell lysates. Amenable to optimization for high-throughput workflows.

Applications

Technology ID

20180317

Category

Engineering & Physical
Sciences/Chemicals
Engineering & Physical
Sciences/Instrumentation,
Sensors & Controls
Life Sciences/Biomarkers
Life Sciences/Research Tools

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- Measurement and comparison of site-specific protein ubiquitination
- Ubiquitination clinical biomarker discovery and assessment
- High throughput ubiquitination measurements

Researchers

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Publications

[*A Quantitative Chemical Proteomics Approach for Site-specific Stoichiometry Analysis of Ubiquitination*](#)

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