Biomarker Assay Development

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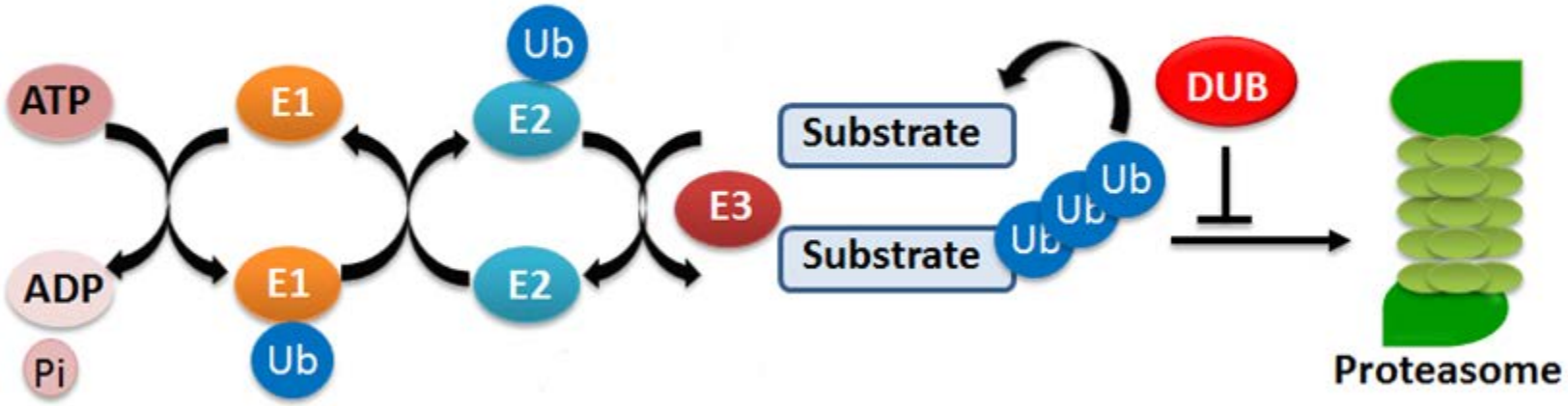
LifeSensors

- Leading Biotech in UPS Drug Discovery and Diagnostic R&D
- ~500 Products, Proteins, Ubiquitin Affinity Reagents (TUBEs), Inhibitors, Assays, Kits and Proprietary Protein Expression Systems (SUMO)
- Drug Discovery, UPS and PROTAC Screening Services
- Profiling Compounds Against Ubiquitin Ligases and DUBs
- Custom Assay Development and Collaborative Research

Importance of Biomarkers

- **Design of clinical studies to determine if a potential drug is a good candidate for the market (clinical assessment)**
- **Prediction of individuals in a population who will develop disease (risk prediction)**
- **Identifying individuals with diseases that are currently asymptomatic (early detection)**
- **Identifying pathways for therapeutic intervention (targeted prevention/therapy)**
- **Identifying risk factors that increase the possibility of disease (risk prevention)**
- **Identifying individuals that will benefit from targeted therapy (personalized medicine)**

Ubiquitin Proteasome System



E1 – Ubiquitin activating enzyme

Requires ATP to attach Ub to E1

E2 – Ubiquitin conjugating enzyme

Transfers Ub from E1 to E3

E3 – Ubiquitin ligases

Transfers Ub to self or substrate

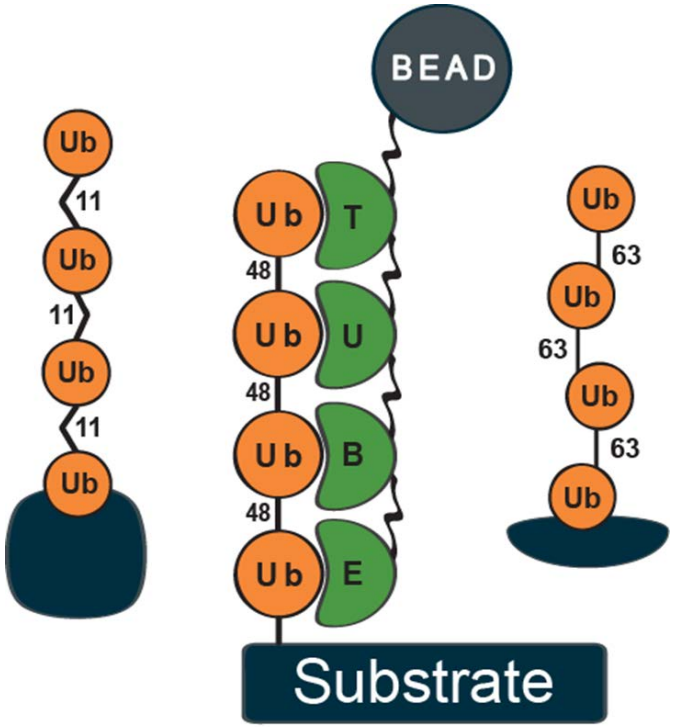
Forms mono-Ub or poly-Ub chains

DUB – Deubiquitinase

Removes mono-Ub or poly-Ub chains

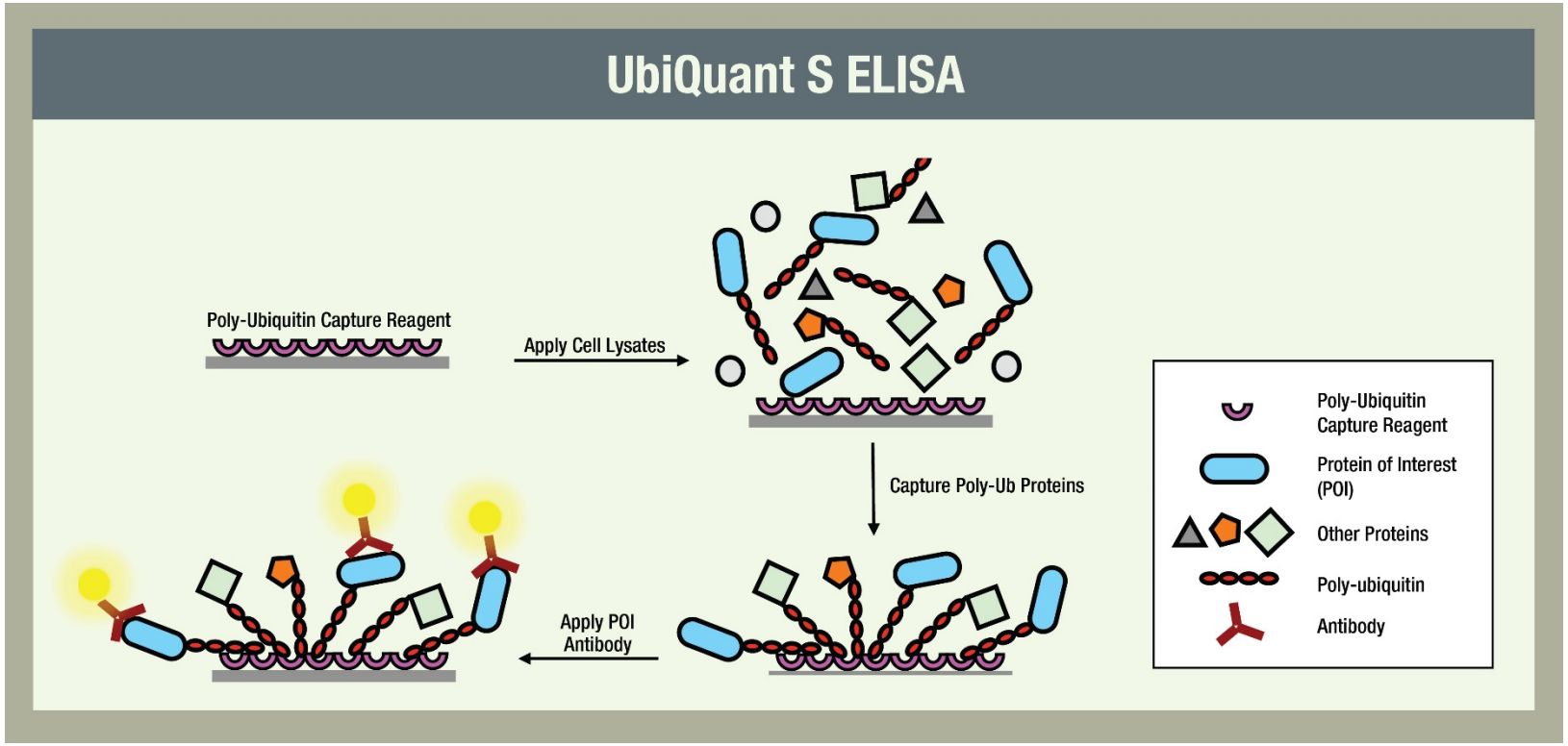
Proteasome – Degrades ubiquitylated proteins

TUBE Properties



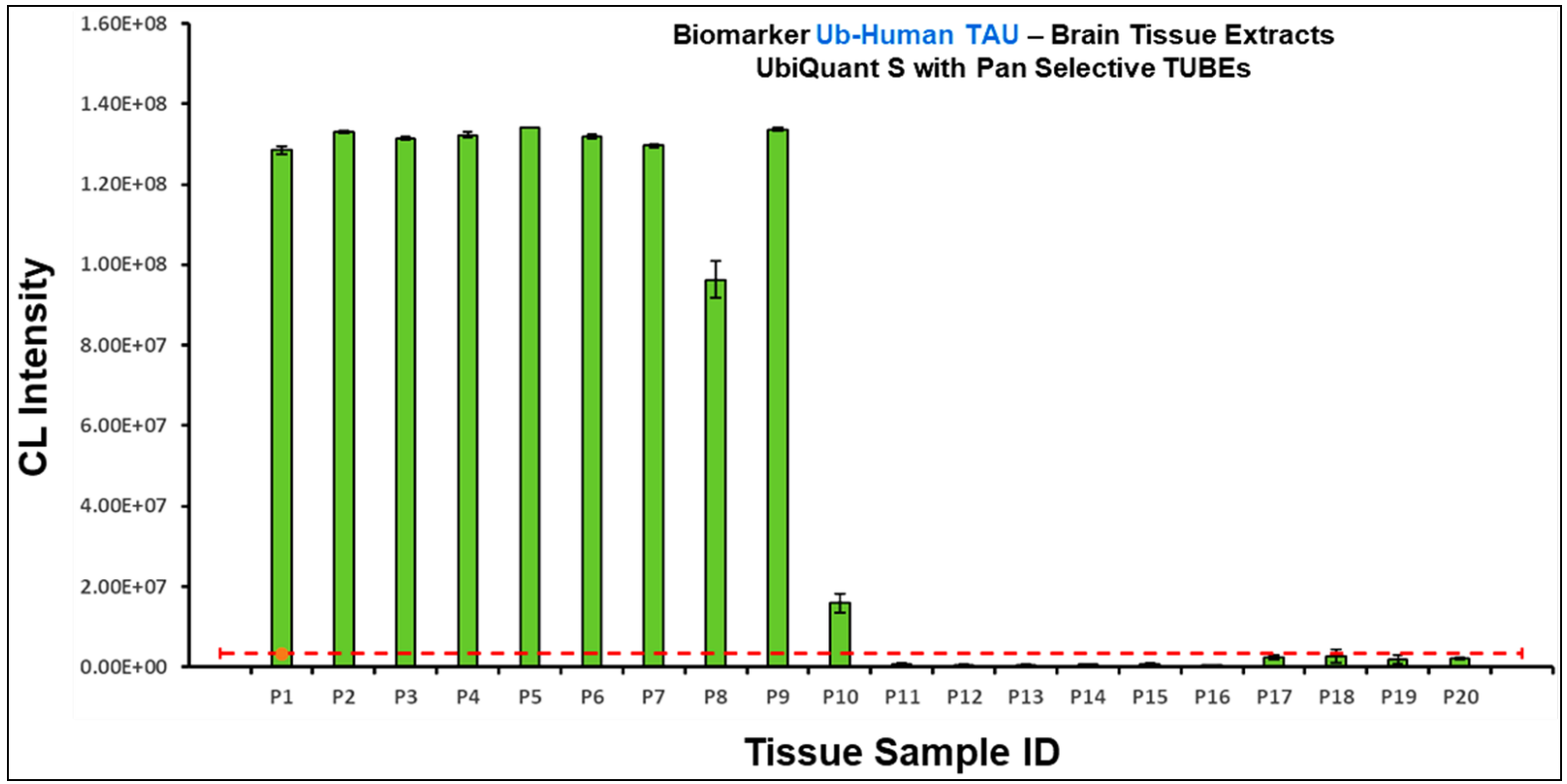
- Natural ubiquitin-binding domains (UBDs)
- Designed and engineered for high affinity and selectivity for polyubiquitin chains
- Superior to antibodies with regards to selectivity and versatile applications
- Labeling with tags (i.e., 6xHis, GST, Biotin, Magnetic, fluorophores, etc.)
- Pan-selective and polyubiquitin chain linkage-selective TUBEs
- Variety of applications, mass spec proteomics, imaging, HTS and biomarkers

A New Platform for High-throughput Biomarker Discovery - UbiQuant S ELISA



In the **UbiQuant S ELISA assay**, polyubiquitylated proteins are captured by an immobilized ubiquitin affinity matrix. Subsequently, unbound proteins are washed away, and ubiquitylated protein of interest bound to the matrix is identified using a POI-specific antibody and traditional ELISA detection reagents.

Example - High-throughput UbiQuant S Assay for Biomarkers in Alzheimer's Disease



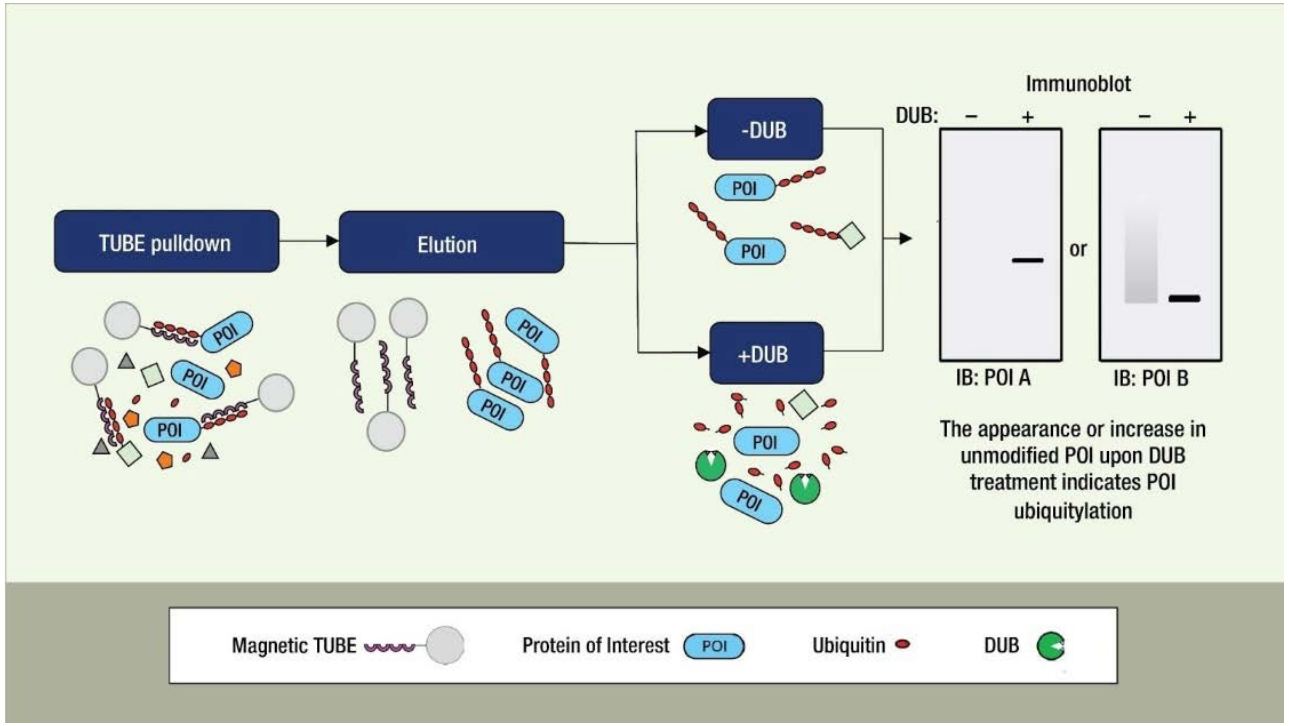
P1-P10
Alzheimer's Patients

P11-P20
Control Patients

- High throughput UbiQuant S assay performed on 10 control and 10 AD brain extracts using Pan-selective TUBEs followed by Human tau antibody probing.
- Results suggest clear differentiation of elevated ubiquitylated TAU between AD cases with different age groups and matched control cases

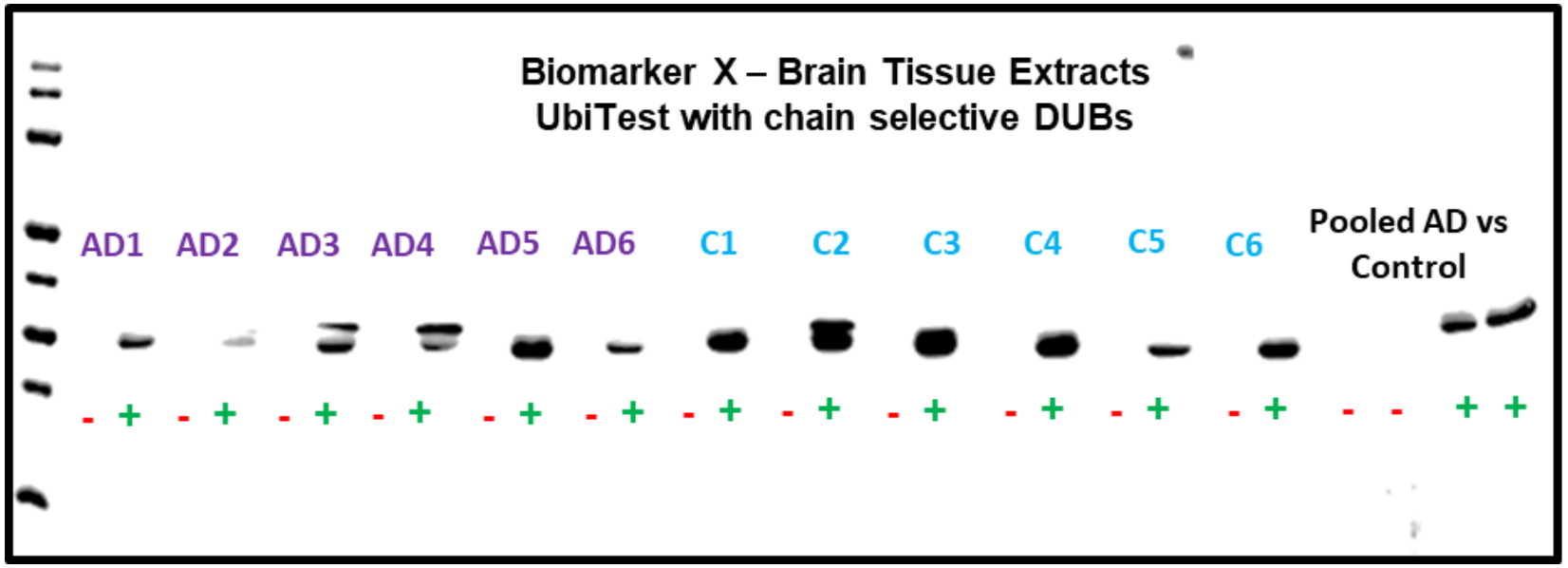
LifeSensors from genomics to proteomics

Chain Selective UbiTest – Assay to Reveal Low Abundant Ubiquitylated Substrates for Discovering Biomarkers



UbiTest is a medium-throughput platform for measuring the polyubiquitylation levels of one or several protein(s) of interest (POI). UbiTest utilizes TUBE technology to enrich for polyubiquitylated proteins. This fraction is analyzed by immunoblotting techniques with and without pan-selective DUB digestion. The appearance or increase in the intensity of the band corresponding to the POI means that the POI is polyubiquitylated in the studied condition.

Example – Chain Selective UbiTest for Biomarkers in Alzheimer's Disease

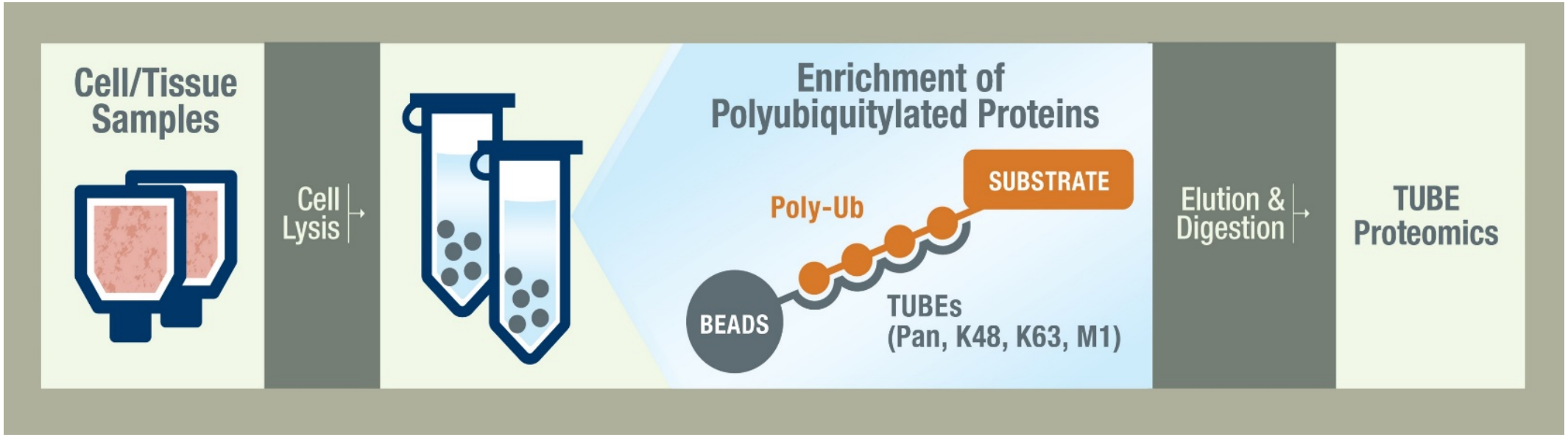


- Immunoprecipitation of polyubiquitylated proteins performed on 6 AD and 6 control brain extracts using pan selective magnetic TUBEs followed by treatment with chain selective DUBs to enrich native low abundant substrates.
- UbiTest analysis shows post DUB treatment enriched novel biomarker with reduced levels in AD patients compared to age matched control patients. ("-" No DUB treatment and "+" DUB treatment)

TUBE-based Mass Spec Ubiquitin Proteomics for Biomarker Discovery

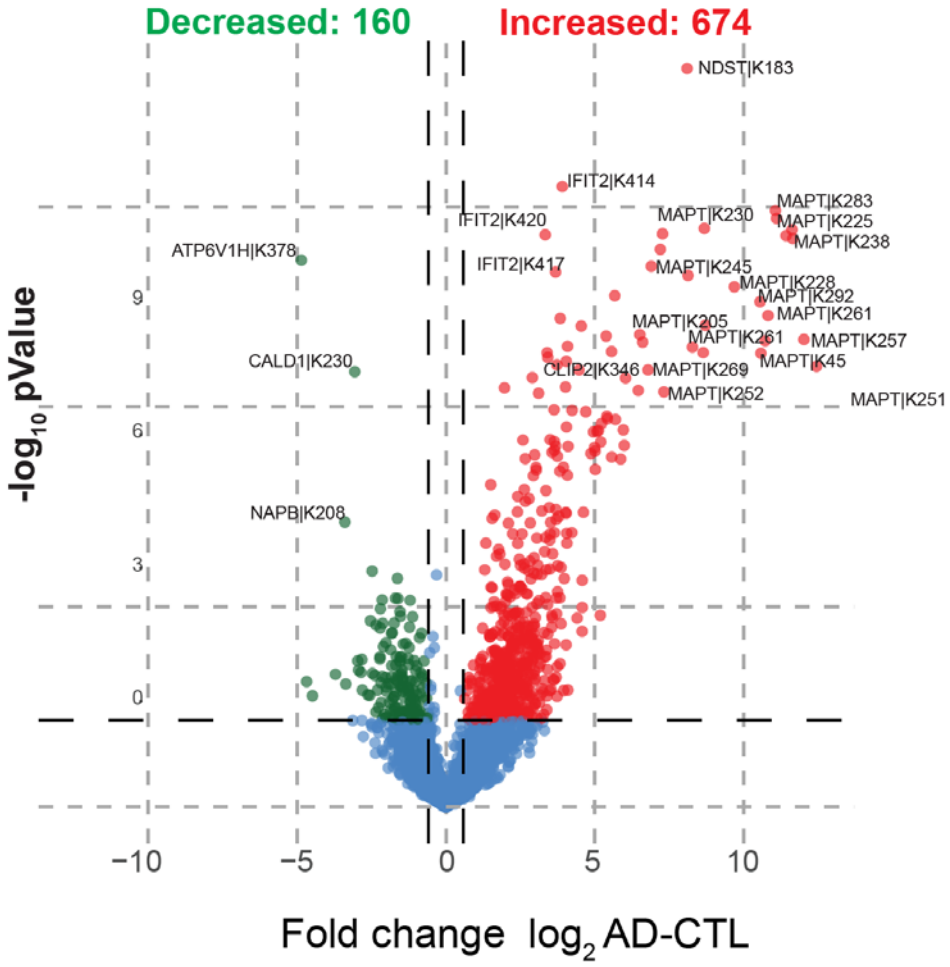
- TUBE applications has simplified ubiquitin proteomics
- Rapid and quantitative analysis of biomarkers from cells and tissues
- Quantitative method for examining drug (DUB, Ligase, PROTAC) effects in cells
- Inexpensive and simple, no need for SILAC or other labeling protocols
- Superior to Di-Gly ubiquitin proteomics

Workflow for TUBE-based Ubiquitin Proteomics



TUBE proteomics is initiated by incubating cell/tissue lysates with TUBEs. After eluting the enriched polyubiquitylated fraction, it is subjected to a tryptic digest followed by MS analysis. LifeSensors provides a comprehensive list in an Excel sheet with all proteins, peptides, and ubiquitylated sites that were detected by MS as well as a quality report summarizing the results of the experiment.

Quantitative TUBE-based Proteomic Analysis of Brains from Alzheimer's Diseases Patients



- Red and green dots represent differentially increased or decreased ubiquitylated peptides in the AD vs. control, respectively.
- Changes reflect possible biomarkers for AD patients

LifeSensors from genomics to proteomics

Biomarker Discovery

- Help customers identify biomarkers specific to drug treatment
- UbiQuant S, a high-throughput assay for ubiquitylated biomarkers
- UbiTest, a medium-throughput assay for ubiquitylation of substrates
- TUBE-based proteomics to identify biomarkers for diseases or drug treatments
- All IP and data belong to the customer
- Work performed under CDA and Master Service Agreement
- Fee for service model, defined milestone-based agreement

Contact Us!

We are your partner for Biomarker Discovery

Contact Information

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