Protein expression profiling with high-content antibody arrays using Universal Linkage System ULS™ protein labeling

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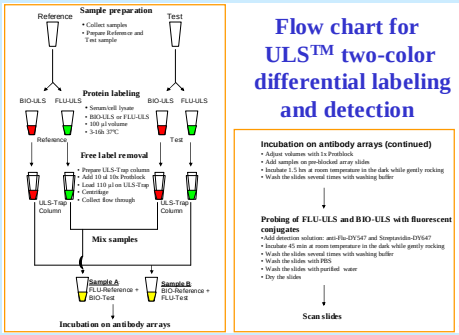
Introduction

- The Universal Linkage System (ULS™) is a platinum-based technology that allows direct labeling of proteins and nucleic acids with a large variety of labels and haptens, including many fluorophores, biotin, and dinitrophenol.
- In proteins, ULS™ forms a stable coordinative bond with the sulfur atom in Methionine and free Cysteine, and with a nitrogen in Histidines (Fig. 1).

ULS™:
- Is an easy and robust labeling method
- Provides high coverage of proteome
- Is stable in aqueous solution
- Labeling is compatible with a wide range of buffers and detergents including Tris, Glycine, SDS, Triton X-100, NP-40 etc.



Flow chart for ULS™ two-color differential labeling and detection

Sensitivity and specificity using ULS™ labeling

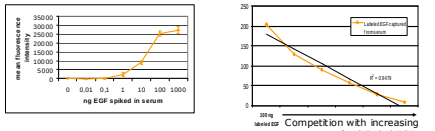
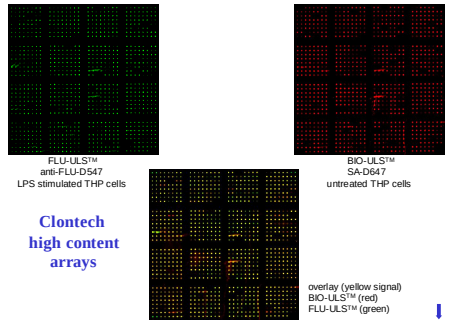


Figure 5. EGF labeling and detection. Left figure: Indicated amounts of EGF were spiked into serum and labeled with BIO-ULS™. Each spiked sample was incubated on a FAST™ array containing monoclonal anti-EGF antibody. Captured BIO-labeled EGF was detected with streptavidin-DY647™. Of note is a dynamic range of 3-4 log, and a sensitivity of high pg/ml detection.
Right figure: 100 ng EGF was spiked into serum and labeled with BIO-ULS™. Subsequently and before array incubation, increasing amounts of "cold" non-labeled EGF was spiked into each one of the samples. Data points show EGF signal obtained for each competition. Of note is the complete competition towards zero indicative for EGF signals only.



ULS™ principle in protein labeling

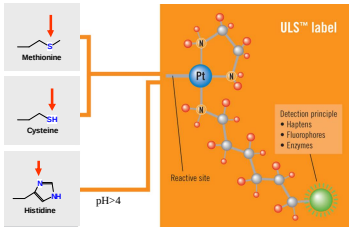


Figure 1. Schematic drawing of a ULS™-label and its protein target sites. ULS™ forms a stable coordinative bond with the sulfur atom in Methionine and Cysteine, and with a nitrogen atom in Histidine (pH>4)

Comparison of ULS™-Trap and gel filtration column specifications

Table 1. Performance of free label removal using ULS™-Trap column versus any given gel filtration column.

	ULS™-Trap	Gel-filtration (any given column)
Free label removal	> 99.5%	94-98%
Principle	Affinity purification => No cut-off	size exclusion => cut-off: 7 kDa
Protein loss	< 1%	10-30%

Two color ULS™ serum profiling (SBC slides) using spiked antigens

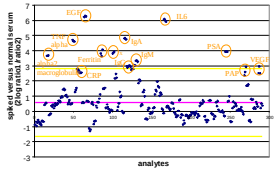
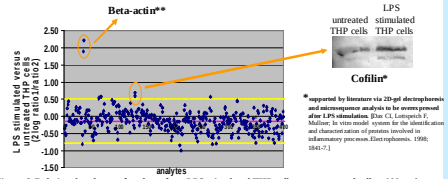


Figure 4. Relative abundance of spiked analytes versus unspiked serum on serum biomarker chip (SBC; Whatman Schleicher & Schuell).
~100 analytes with corresponding antibodies spotted in triplicate were analyzed in a two-color differential BIO/FLU-ULS™ labeling approach with label swap for relative expression of spiked analytes. Purple line represents mean of all abundance ratios. Yellow lines represent 2*SD away from mean abundance ratio. Spiked analytes are highlighted in orange. Note that one antibody showed cross-reactivity (haptoglobin= x) after spiking with antigens.

Two-color ULS™ cell lysate labeling validation after antibody array profiling



* supported by literature via 2D-gel electrophoresis and microsequence analysis to be overexpressed after LPS-stimulation. (Das CI, Lottspeich F, Mullner; In vitro model system for the identification and characterization of proteins involved in inflammatory processes. Electrophoresis. 1998; 1841-7.)

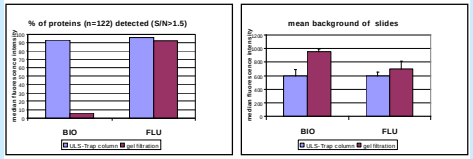
Figure 6. Relative abundance of analytes from LPS stimulated THP cells over untreated cells. ~200 analytes with corresponding antibodies spotted in duplicate on Sigma Panorama™ arrays were analyzed in a two-color differential ULS™ labeling approach for relative expression between untreated and LPS stimulated THP cells. Purple line represents mean of all abundance ratios. Yellow lines represent 2*SD away from mean abundance ratio.
** Beta-actin not validated yet. **All cell lysate experiments performed in collaboration with NV Organon.**

ULS™ labeling data presented

In this poster we present ULS™ labeling data that support its suitability for antibody array profiling.

- ULS™ is suitable for two-color differential display on high-content arrays
  thus no need for validated antibody pairs like in sandwich approaches
- ULS™ efficiently labels samples from different origin
  e.g. serum and cell lysate
- ULS™ is compatible with arrays with different surface chemistries
  e.g. nitrocellulose-based slides, epoxysilane-coated slides
- Compatible with commercially available high-content arrays
  e.g. with slides and protocols from Sigma (Panorama™), Clontech, and Whatman (SBC) antibody arrays kits

ULS™-Trap column is essential for optimal antibody microarray performance



A B

Figure 3. Antibody array performance after free label removal with ULS-Trap and gel filtration. **(A)** % of analytes detected in human serum with a minimal signal to noise ratio of 1.5, labeled with BIO-ULS or FLU-ULS and probed on a serum biomarker chip (SBC) slide from Whatman after regular gel filtration or using an ULS-Trap column. **(B)** calculated mean background of probed slides comparing gel filtration and ULS-Trap column.

ULS™ cell lysate labeling and performance on high content antibody array

	Assay performance	
	Assay 1	Assay 2 (reproduction)
INR* (non-differentials)	0.77-1.3	0.8-1.27
% spots fitting the criteria**	87.5%	91.5%

Table 1. Same versus same data using two color cell lysate labeling on Clontech high content (512) antibody microarray

*INR= internalized normalization ratio

$$INR = \sqrt{\frac{Bio_{sample}}{Bio_{reference}} \times \frac{Flu_{sample}}{Flu_{reference}}}$$

**CRITERIA FOLLOWED 1: intra-slide spot variation not exceeding 20%
2: inter-slide spot variation not exceeding 30%
3: sg/bg >1.5
4: normalization via mean log2 ratio/ratio of all spots

Assay performance	Mean values of all spots	
	BIO-ULS (PMT gain 600)	FLU-ULS (PMT gain 500)
CV (%)	21	21
signal/ background	13.9	16.3
net signals	5600	9200
Background	450	600

Table 2. Specifications of BIO-ULS™ and FLU-ULS™ labeling and detection on Clontech antibody microarrays

ULS™ antibody array performance

- Efficient labeling of complex protein mixtures
- Non-selective protein labeling
- Compatible with many types of samples
  e.g. serum and cell lysate
- Compatible with many types of lysis conditions and washing/blocking buffers
  e.g. Sigma Panorama™ and Clontech array kits
- Reproducible
- 3-4 Log dynamic range
- High signal/noise ratios
- Sensitive (serum: high pg/ml, low ng/ml range)
- Compatible with many array surfaces
  e.g. nitrocellulose- (FAST™, PATH™, Panorama™) and hydrogel-based slides (Optarrays™) , amino-silane and epoxy-silane slides, CodeLink™ slides.

ULS™ in conclusion

➡ ULS™ is the method of choice for serum/cell lysate labeling for biomarker profiling on high-content antibody arrays