

# Smart Protein Layers

## Quick Guide Protein Labeling

### 1 Required materials for SPL-labeling

- Smart Label Working Solution (SLW)
- SPL Buffer
- SPL Smartalyzer (SMA)
- 60 mM DTT (newly prepared)
- Calibrator-Mix
- Sample with a concentration of ca. 5 µg/µl

(for concentrations lower than 1 µg/µl please contact the customer support)

### 2 Preparation of the SLW (only before first usage)

1. Allow vials containing Smart Label reagent A and B to warm up to room temperature and spin down briefly.
2. To solubilise the Smart Label add 15 µl of Smart Label reagent B to Smart Label reagent A and mix. Spin down briefly. Note: Reagent B contains molecular sieves to avoid water rehydration.
3. Transfer all liquid from Smart Label reagent A to Smart Label reagent B, mix and spin down briefly.
4. The SLW solution is now ready for usage and stable for 6 months.

Store the SLW solution at -20 °C to -80 °C

### 3 Preparation of the Calibrator (CAL) as a Master Mix (recommended)

1. Transfer 8 µl CAL A per gel into a fresh micro-centrifugation tube.
2. Add 2 µl CAL B per gel and mix.  
Note: For low abundant Western Blot target proteins we recommend to dilute CAL B.
3. Add 2 µl of 60 mM DTT per gel (newly prepared), mix and centrifuge briefly.
4. Denature the the proteins by heating the mixture for 4 min at 95 °C.
5. Apply 12 µl of the prepared CAL to each gel.

# Smart Protein Layers

## Quick Guide Protein Labeling

### 4 Preparation of the Reaction and Loading Mix (RL Mix)

6 µl	SPL Buffer	} x (n*+1)	* sample number
2 µl	60 mM DTT		
2 µl	SMA (S or L)		

#### Note:

The SMA and CAL B standards can be used in dilution if the fluorescent signal is too prominent.

Pre-dilute SMA and CAL B with water or PBS before preparing the RL or CAL Mix (e.g. 1:100).

The total volumes used for RL Mix or the CAL Mix should not be altered.

### 5 SPL Labeling

Variant A) for equal sample volumes with different protein concentrations

protein conc.	sample vol.	H <sub>2</sub> O**	RL-Mix	SLW	denaturing	gel load
up to 10 µg/µl	10 µl	-	10 µl	1 µl	5 min, 95 °C	21 µl

Variant B) for equal protein concentrations in different sample volumes (e.g.)

50 µg	4 µl	6 µl	10 µl	1 µl	5 min, 95 °C	21 µl
50 µg	9 µl	1 µl	10 µl	1 µl	5 min, 95 °C	21 µl

\*\* or buffer in which sample is solved

For an excel sheet ask the Service Team ([info@dyeagnostics.com](mailto:info@dyeagnostics.com))