

Jess™ : high throughput capillary western

Unil

UNIL | Université de Lausanne



All steps of traditional western blotting are fully automated inside capillaries



- 12 or 24 independant samples/run
- Different antibodies in the same run
- 72 samples in 24h on one or more targets
- Separation 2-44 kDa
12-230 kDa
66-440 kDa

Regular run

Load matrix and samples

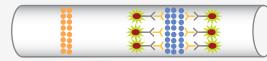


3h

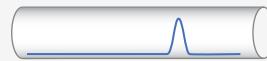
Separate and immobilize



Antibody 1 immunoprobe



Detect and quantify target 1



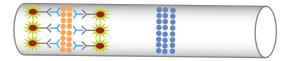
RePlex run

If desired, a second run could be performed within the same capillary.

Remove antibody from 1st immunoprobe



Antibody 2 immunoprobe (or Total Protein Assay)



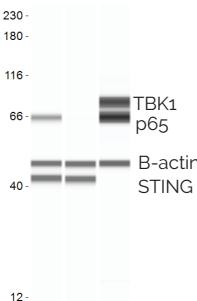
Detect and quantify target 2



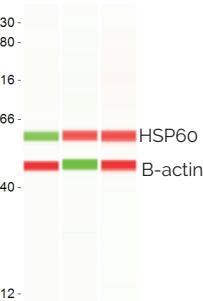
Adapted from ProteinSimple

3 detection modes for multiplexing

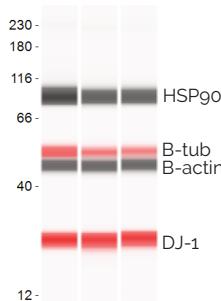
Chemi luminescence



Fluorescence



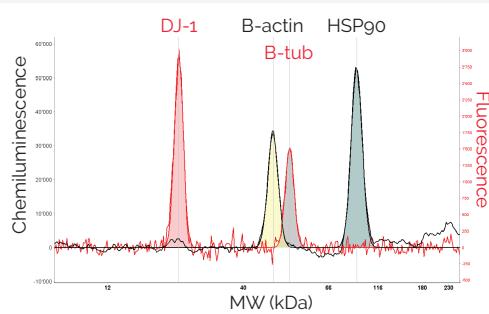
Chemi + fluo



Accurate quantification

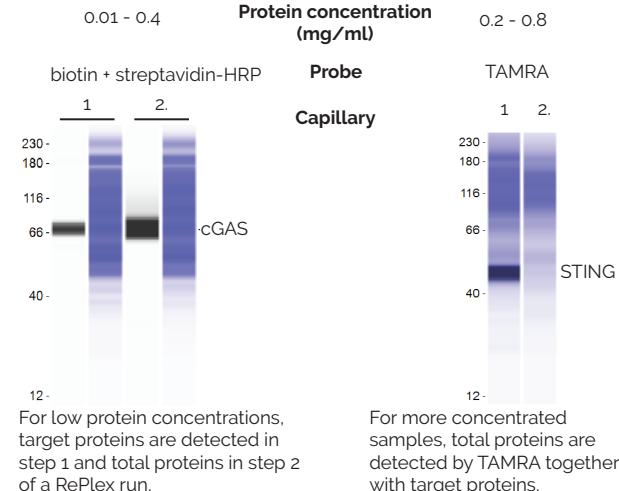
Area under the curve for precise and relative quantification.

Intra-run CV <10%
Inter-run CV < 20%



Total protein normalisation

More reliably than housekeeping proteins, data can be normalized to total proteins loaded into the capillary.



For low protein concentrations, target proteins are detected in step 1 and total proteins in step 2 of a RePlex run.

For more concentrated samples, total proteins are detected by TAMRA together with target proteins.

How to proceed

1

Discussion

Background, samples, antibodies

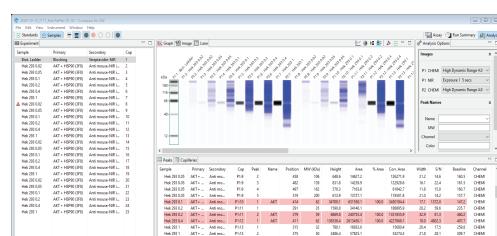
2

Test run

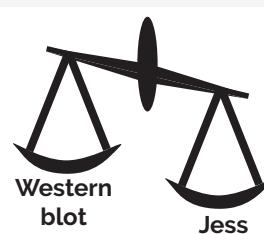
- 15 µg protein
- 5 µl antibody

3

Run(s) Raw file Analysis



Linear range
Antibodies saturating conditions
Multiplexing
Normalisation



Western blot

Jess

- Fast
- Flexible
- Accurate
- Quantitative
- Reproducible
- Less material and antibodies