Accessing precious samples in Western blots by capillary electrophoresis interfaced to membrane via inkjet

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Diabetes & Islets of Langerhans

Diabetes

>8% of adult population diagnosed

~1/3 of US adults are prediabetic

7th leading cause of death

Risk factors include: age, weight, race, family history, high blood pressure, other health factors

Can lead to: heart/kidney/dental disease, nerve damage, blindness, stroke, foot problems

Figure courtesy of Ashley Lenhart
Conventional Western blot

**Advantages**
- Robust and widely used
- Good specificity and sensitivity
- Semi-quantitative

**Disadvantages**
- Large sample and reagent use
- Long analysis times
- Manual operation

Electrophoresis: 1 – 50 µg, ~1 h
Electroblot: 1 h - overnight
Immunoblot: 6 h - overnight
Capillary electrophoresis with inkjetting (CEI)

Sciex SDS-MW gel buffer

FASI injections: samples at ~1 µg/mL

10 cm, 40/150 ID/OD
Title: Field strength

Graphs illustrate the relationship between field strength and various parameters such as time, resolution, peak height, and stage speed. The graphs show the impact of different field strengths (300 V/cm, 350 V/cm, 400 V/cm) on these parameters over time and stage speed.
Separation repeatability

CEI Ladder Repeatability

Conventional Ladder Repeatability
Limit of detection

Actin LOD by Conventional

- LOD = 250 pg / 300 pM

y = 0.9386x + 8.132
R^2 = 0.9845
n = 1

Actin LOD by CEI

- LOD = 14 pg / 6 pM

y = 1.144x - 97.97
R^2 = 0.9710
n = 1
Islets of Langerhans

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**ERK1/2**

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**GSK-3β**

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**4E-BP1**

12 samples – 45 islets

14 samples – 4 islets
Islets of Langerhans

Islet Isolation
- Surgery performed in-lab
- Islets picked by hand
- Multi-hour process
- ~200 islets extracted

Conventional
- 1 – 50 µg per sample (1-50 islets)
- 4 – 200 samples per mouse

CEI
- 100 ng per sample (0.1 islet)
- 2000 samples per mouse
Acknowledgements

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- Dr. Natalie Arvin
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