Technical Note

Detection limit of the FastGene® Western ECL kit used for whole-cell lysates

Product	FastGene® Western ECL kit (FG-CH01)
Manufacturer	NIPPON Genetics EUROPE GmbH

The following data was provided by the manufacturer: NIPPON Genetics EUROPE GmbH

Purpose

Evaluation of the detection limit of FastGene® Western ECL Kit as chemiluminescence substrate for western blot analysis and detection of house-keeping genes from whole-cell lysates. In addition, detection sensitivity for primary antibody incubation for 1h at RT or overnight at 4°C was compared.

Summary

FastGene® Western ECL Kit is an enhanced chemiluminescence (ECL) substrate based on luminol. It is used to detect horseradish peroxidase (HRP) – conjugated secondary antibodies. The high femtogram or low picogram detection of antigens is enabled by FastGene® Western ECL Kit brilliant sensitivity and long signal duration.
This technical note shows the evaluation of the detection limit of FastGene® Western ECL Kit used for western blot analysis. Results show, that it provides a sensitive method to detect specific protein bands of purified protein in as low as 80 ng (0.08 µg) whole-cell lysates. Furthermore, antibody-incubation overnight (4°C) can significantly increase the detection sensitivity compared to incubation for 1 h (RT).
The use of the FastGene® Western ECL Kit is a powerful tool for detection of low concentrated proteins, thereby saving valuable protein samples.

Reagents

- FastGene® Western ECL Kit
- TBS-0.1%, Tween-20 (TBST)
- 5% non-fat dried milk in TBST
- Antibodies: Rabbit α-β-Actin (1:1000 dilution)
  Mouse α-Vinculin (1:5000 dilution)
  Goat α-rabbit-HRP (1:10.000 dilution)
  Goat α-mouse-HRP (1:10.000 dilution)

Experimental procedure

Whole-cell lysate:

Whole-cell protein lysate was isolated from HEK 293 cells. Cells were incubated with Triton lysis buffer (50 mM Tris, 150 mM NaCl, 1 % Triton-X 100, 1 mM DTT) for 30 min and supernatant (= protein lysate) was collected for further analysis.

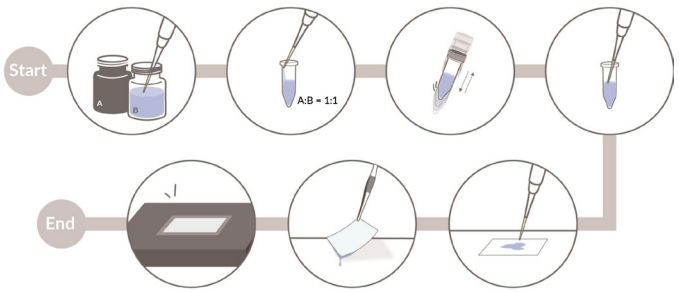
Western blotting:

5 to 0.015 µg in a 2-fold serial dilution row were separated using a FastGene® PAGE Gel 8-16 % (120 V, ~1.5 h) and blotted to a PVDF membrane using the Trans-Blot Turbo Transfer System (Bio-Rad). Western Blots on PVDF membrane were cut at the 70 kDa marker band and blocked with 5 % non-fat dried milk in TBST for 1h at RT. Subsequently, membranes were incubated with primary antibodies against Vinculin (116 kDa) or β-Actin (45 kDa), diluted in 5 % non-fat dried milk in TBST, for 1 h at RT or overnight at 4°C (o.n.). Blots were washed three times (5 min each) with TBST and incubated with HRP-coupled secondary antibody dilutions (in 5 % non-fat dried milk in TBST). The membrane was washed with TBST for three-times (10 minutes each) before detection.

Western blot development:

Subsequently, development was performed using FastGene® Western ECL Kit according to the manufacturer's instructions:

1. Mix Luminol solution and Peroxide Solution in a 1:1 ratio, and thoroughly agitate the chemiluminescent substrate solution well. Prepare 0.1 ml of solution/cm² of membrane.
2. Place the membrane with the protein side up on a clear and level surface or in a clean container.
3. Evenly distribute the prepared chemiluminescent substrate solution on the membrane. Make sure, the whole membrane area is covered.
4. Incubate membrane for 1 min
5. Remove the membrane from the chemiluminescent substrate solution and drain off excessive solution.
6. Place the membrane in a plastic sheet protector or in plastic wrap to prevent the membrane from drying.

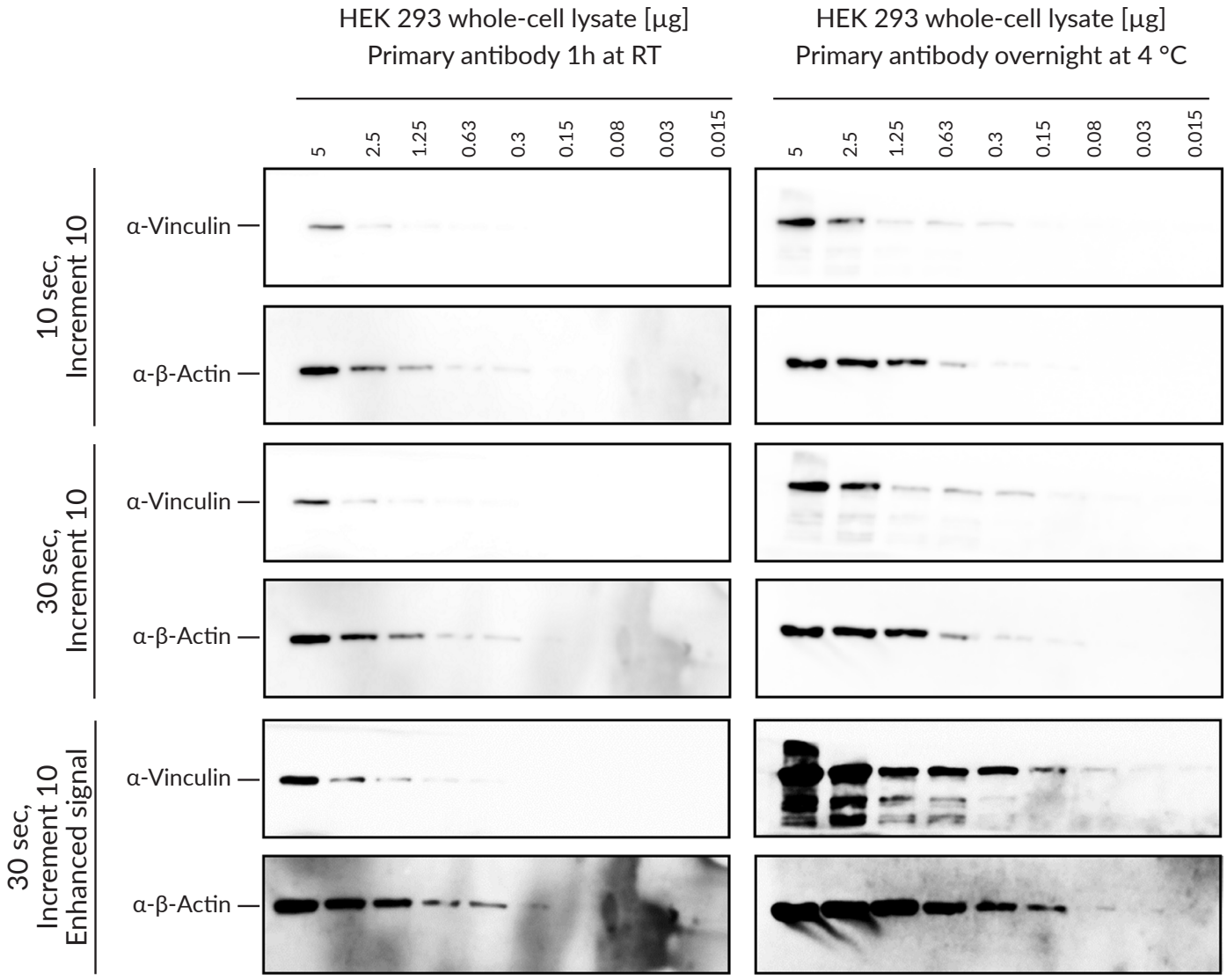


Detection workflow used for comparison of the FastGene® Western ECL Kit

Afterwards, signal detection was performed using an Azure 400 Visible Fluorescent Western System (Azure biosystems).

Results

Detection of protein signal was performed using the FastGene® Western ECL kit according to the manufacturer's instructions and a Azure 400 Visible Fluorescent Western System (Azure biosystems). Signal detection was performed as increment using exposure times of either 10 or 30 seconds. Azure software was used to enhance the signal intensity (enhanced signal) for better visibility of low concentrations.



Conclusion

The detection sensitivity of the FastGene® Western ECL kit is very high and as low as 300 ng of whole-cell lysate can be loaded to easily detect abundant proteins. To further increase the detection sensitivity, incubation with primary antibodies overnight at 4 °C is recommended. Here, the detection limit can be improved to 80 ng input.

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