Technical Note

Detection sensitivity comparison of the FastGene® Western ECL kit

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 FastGene® Western ECL Kit as a chemiluminescent substrate for Western blot analysis compared to the manufacturer's competing substrates.

Summary

The FastGene® Western ECL Kit is a luminol-based enhanced chemiluminescence (ECL) substrate. It is used for the detection of horseradish peroxidase (HRP)-conjugated secondary antibodies. Detection of antigens in the high femtogram or low picogram range is enabled by the brilliant sensitivity and long signal duration of the FastGene® Western ECL Kit. This technical note demonstrates the evaluation of the FastGene® Western ECL Kit for Western blot analysis. In addition, the FastGene® Western ECL Kit was compared to antigen detection using the Clarity™ Western ECL Substrate (Bio-Rad) and the Pierce™ ECL Western Blotting Substrate (Thermo Fisher). The results show that the FastGene® Western ECL Kit is a sensitive method for detecting specific protein bands from purified protein with a higher sensitivity than competing products. The use of the FastGene® Western ECL Kit therefore saves valuable protein samples due to the low detection limit on the one hand and shortens the very time-consuming Western blot detection procedure on the other hand.

Reagents

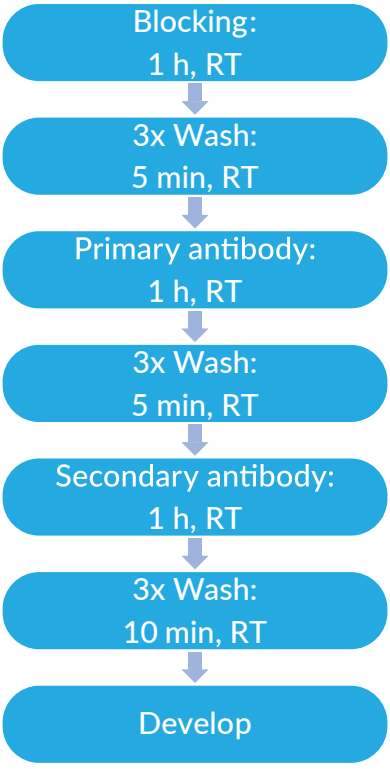
- FastGene® Western ECL Kit
- 5% non-fat dried milk in TBS, 0.1 % Tween-20
- TBS, 0.1 % Tween-20
- Antibodies: Rabbit α-BSA
  Goat α-rabbit-HRP

Experimental procedure

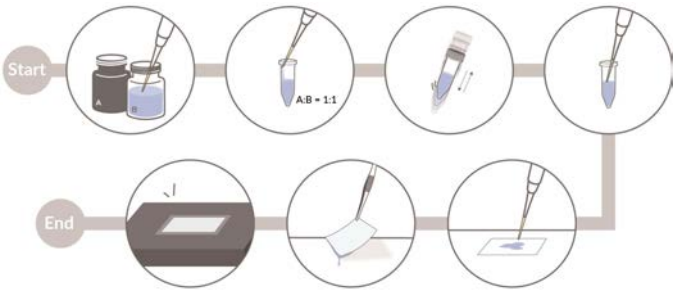
100 to 1000 ng of purified bovine serum albumin (BSA) was separated using a FastGene® PAGE Gel 4-20% and blotted onto a PVDF membrane using the Trans-Blot Turbo Transfer System (Bio-Rad). Western blots on the PVDF membrane were incubated with the appropriate primary (1:5,000) and secondary (1:10,000) antibody dilutions. The membrane was washed three times with TBS-0.1% Tween-20 for 10 minutes before detection.

Subsequently, development was performed using either the FastGene® Western ECL Kit, Clarity™ Western ECL Substrate (Bio-Rad), or Pierce™ ECL Western Blotting Substrate (Thermo Fisher) according to the manufacturer's instructions:

1. Mix the luminol solution and the peroxide solution in a 1:1 ratio and shake the chemiluminescent substrate solution thoroughly. Prepare 0.1 ml of the solution/cm^2 of the membrane.
2. Place the membrane with the protein side up on a clear and flat surface or in a clean container.
3. Spread the prepared chemiluminescent substrate solution evenly on the membrane. Make sure that the entire membrane surface is covered.
4. Incubate the membrane for 1 minute (FastGene®, PierceTM) or 5 minutes (ClarityTM).
5. Remove the membrane from the chemiluminescent substrate solution and allow excess solution to drip off.
6. Place the membrane in a plastic sheet or plastic wrap to prevent the membrane from drying out.
7. Afterwards, signal detection was performed using an Azure 400 Visible Fluorescent Western System (Azure biosystems).



Workflow for western blot analysis

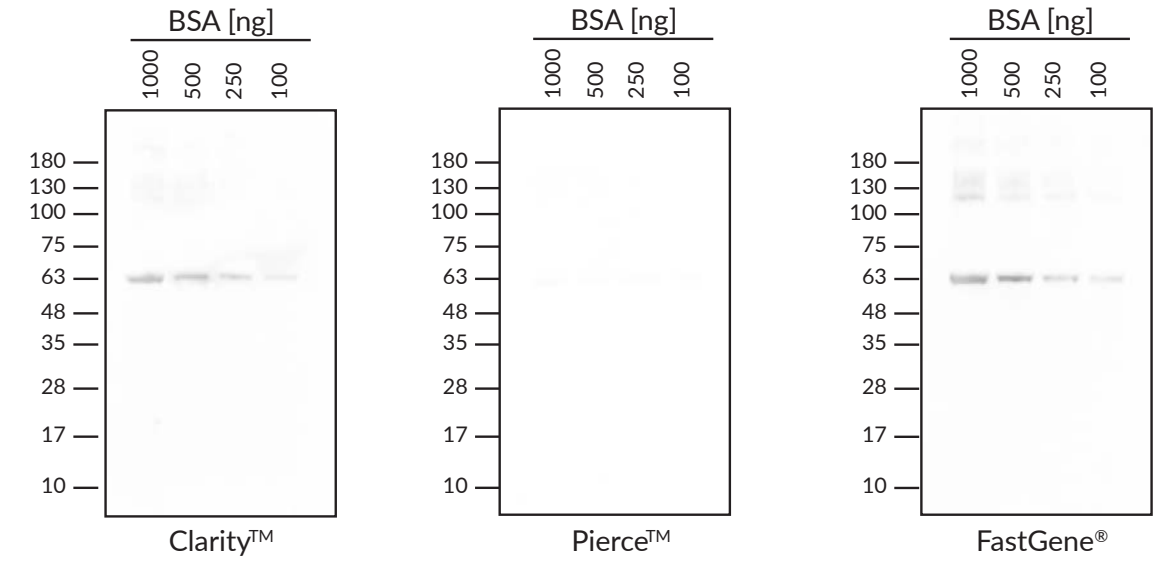


Detection workflow used for comparison of the FastGene® Western ECL Kit

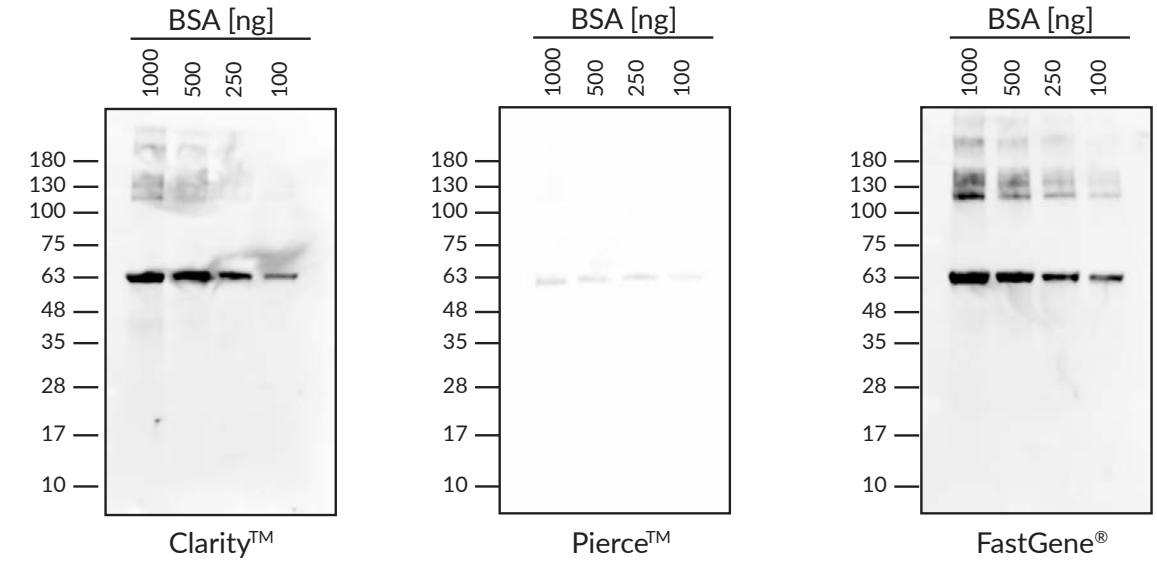
Results

Detection of the protein signal was performed using the three different ECL substrate kits according to the manufacturer's instructions and an Azure 400 Visible Fluorescent Western System (Azure biosystems) with an exposure time of 5 and 10 seconds.

Exposure time 5 seconds:



Exposure time 10 seconds:



Conclusion

The detection sensitivity of the FastGene® Western ECL Kit is very high compared to its competitors Clarity™ and Pierce™. Due to the high sensitivity, the detection time can be significantly shortened and an exposure time of 5 seconds allows reliable detection of even small amounts of protein.

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