



## Technical Note

# Western blot blocking performance of the blocking reagent FastGene® Block & Go

Product

FastGene® Block & Go (FG-CH05)

Manufacturer

NIPPON Genetics EUROPE GmbH

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



## Purpose

Evaluation of FastGene® Block & Go as blocking reagent for western blot analysis of human protein expression in comparison to the standard protocol using 5 % non-fat dried milk as blocking solution.

## Summary

FastGene® Block & Go is a protein-free blocking solution for western blot analysis, additionally enhancing band intensity when developed with HRP (horseradish peroxidase) or AP (alkaline phosphatase) substrates. It can be used in a single step protocol, combining blocking, primary and secondary antibody hybridization, and provides a time-saving method for sensitive detection of protein expression.

This technical note shows the evaluation of FastGene® Block & Go used for western blot analysis in a single versus a two step protocol. Furthermore, FastGene® Block & Go was compared to a standard western blot analysis protocol using 5 % non-fat dried milk. Results show, that FastGene® Block & Go provides a sensitive method to detect specific protein bands in as low as 0.5 µg of whole-cell protein lysate, whereas the common method using milk was only able to detect specific protein expression in amounts of 5 µg or more whole-cell protein lysate. Therefore, the use of the FastGene® Block & Go on the one hand saves valuable protein samples due to the low detection limit and on the other hand shortens the very time- and labor-intensive western blot procedure.

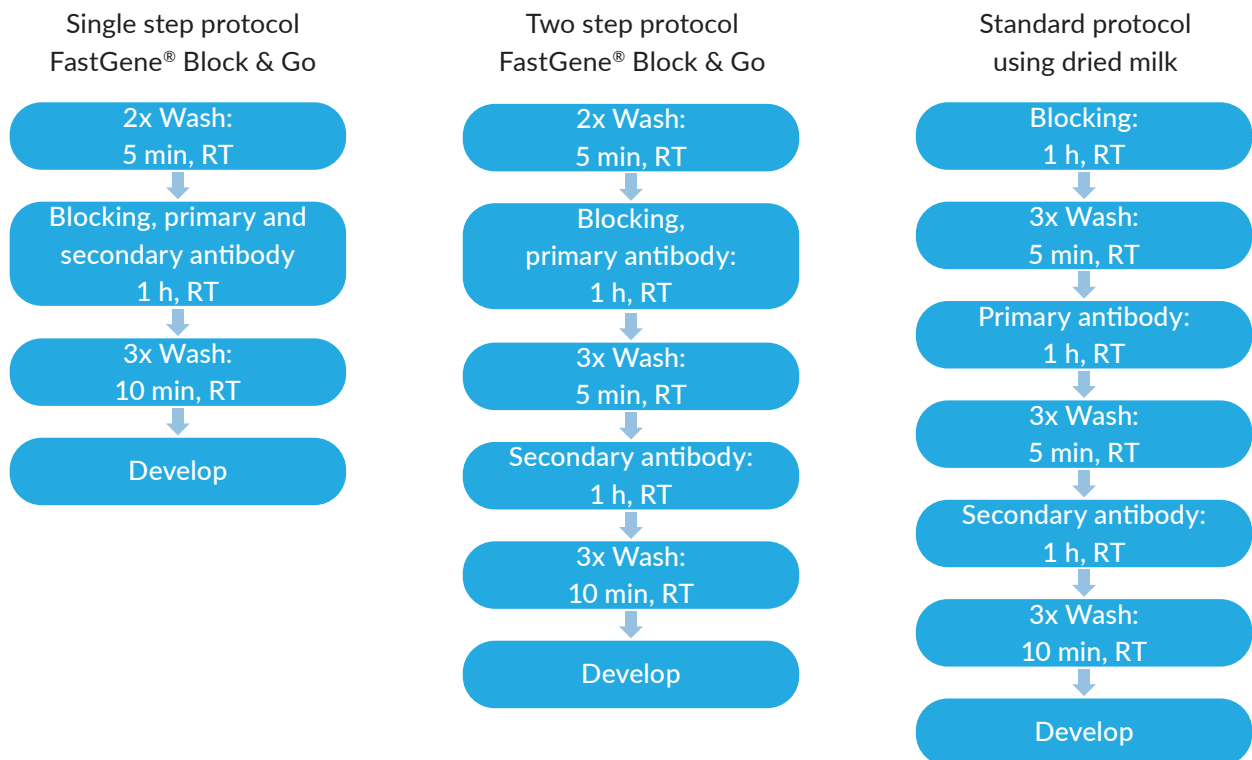
## Reagents

- 5 % non-fat dried milk in TBS-0.1 % Tween-20
- TBS-0.1 % Tween-20
- HEK293 whole-cell lysate
- FastGene® Western ECL Kit
- Antibodies: Mouse  $\alpha$ -human vinculin  
Rabbit  $\alpha$ -human GAPDH  
Goat  $\alpha$ -rabbit-HRP  
Goat  $\alpha$ -rabbit-HRP

## Experimental procedure

0.5 to 10  $\mu$ g of HEK293 whole-cell protein lysate were separated using a FastGene® PAGE Gel 4-12 % and blotted to a PVDF membrane using the Trans-Blot Turbo Transfer System (Bio-Rad). Western blots on PVDF membrane were analyzed using the following protocols:

- Single step protocol FastGene® Block & Go
- Two step protocol FastGene® Block & Go
- Standard protocol using dried milk

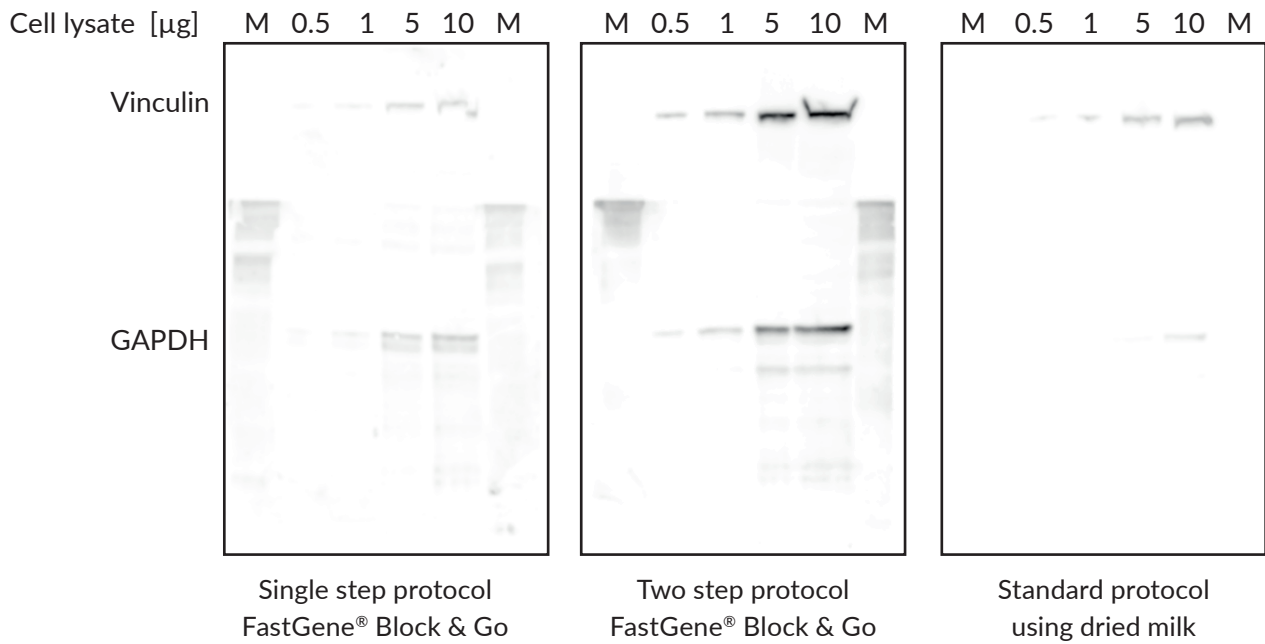


Developing was performed using FastGene® Western ECL Kit according to manufacturers instructions and an Azure 400 Visible Fluorescent Western System (Azure biosystems).

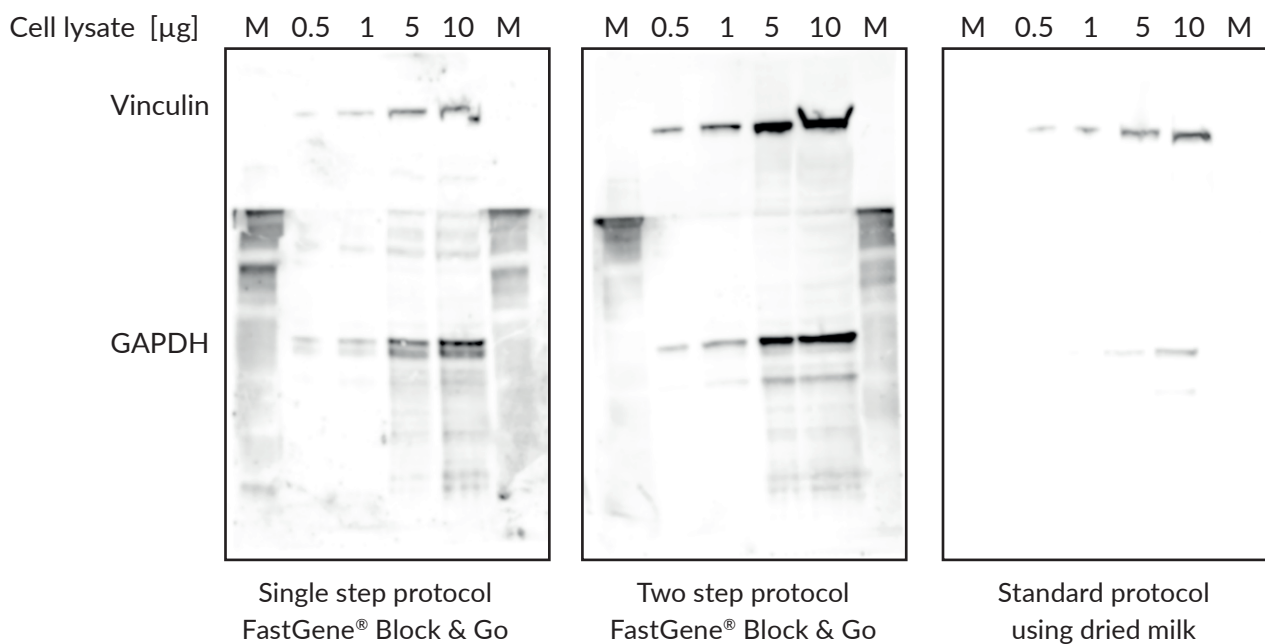
## Results

Detection of protein signal was performed using FastGene® Western ECL Kit according to manufacturer's instructions and a Azure 400 Visible Fluorescent Western System (Azure biosystems).

Exposure time 30 seconds:

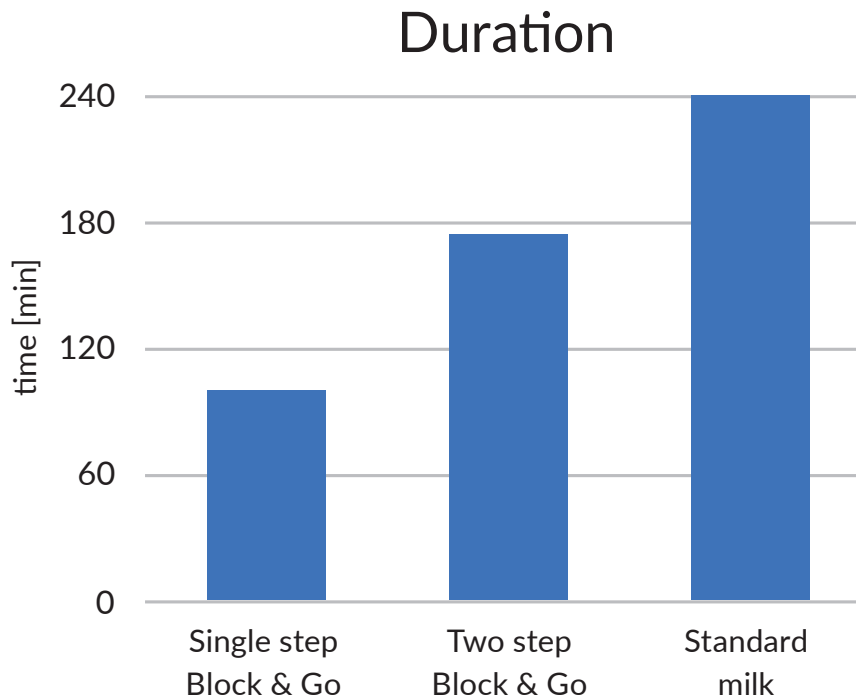


Exposure time 2 minutes:



## Conclusion

FastGene® Block & Go provides a sensitive method for detection of specific protein expression using as low as 0.5 µg whole-cell lysate. In contrast, the standard protocol using 5 % milk has a detection limit of ~5 µg protein lysate.



The standard Western blot protocol using milk has a duration of ~4 h, whereas the use of FastGene® Block & Go can save up to 2:20 h (single-step) and a minimum of 1 h (two-step) of precious work time.