JUL 17, 2018

Western blotting

PLOS One

Yuan Yao¹

¹Inner Mongolia People`s Hospital

Yuan Yao

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.qaddsa6

External link:

<https://doi.org/10.1371/journal.pone.0200688>

Protocol Citation: Yuan Yao 2018. Western blotting.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.qaddsa6>

MANUSCRIPT CITATION:

Yao Y, Ma Y, Chen X, Bade R, Lv C, Zhu R (2018) Absence of RstA results in delayed initiation of DNA replication in *Escherichia coli*. PLoS ONE 13(7): e0200688. doi: [10.1371/journal.pone.0200688](https://doi.org/10.1371/journal.pone.0200688)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: May 22, 2018

Last Modified: Jul 17, 2018

PROTOCOL integer ID:
12325

- 1 Incubate E.coli cells 1:100 in 10 ml LB medium with antibiotics.
- 2 OD600=0.4 , collect all cells at 4 °C, 8000 rpm for 10 min.
- 3 Wash with 1 ml 1*PBS one time.
- 4 Resuspend in 500 ul 1 * PBS and sonicate.
- 5 Spin at 10000 rpm for 15 min.
- 6 Transfer supernatant into a new 1.5 ml tube and sit on ice.
- 7 Load into 120% SDS-PAGE gel , run at 200v for 45 min.
- 8 Get the PVDF membrane and filter paperPVDF: Put in methanol (5s) then in transfer bufferFilter: transfer bufferGel: transfer buffer

- 9 Sit on Room Temperature for 20 min.
- 10 Transfer membrane at 25 V for 30 min.
- 11 Blook woth 5% skim milk RT for 1 hour.
- 12 1:1000 Ab1 DnaA Ab.
- 13 4 °C over night rotation.
- 14 Wash the membrane with 1* PBST for 3 times (15 min , 10 min, 5 min).
- 15 Dillute AB2 1:10000 in 50 ml PBST.
- 16 Incubate with Ab2 for 1 h.

- 17** Wash the membrane with 1* PBST for 3 times (15 min , 10 min, 5 min).
- 18** Mix solution 1 and 2 (1:1).
- 19** Incubate the membrane with Mix solution for 5 s.
- 20** Develop the membrane with ChemiDoc MP Imaging system (BIO-RAD).