

Half Hour Western Blot Kit (JZ-009)

Highlight:

- The time saving:** this kit was designed to help you get your Western blot result quick and easily. It only takes half hour to replace the 5-6 hours or overnight processing on the typical Western Blot.
- Good result:** Easy, quick to get desired result; See working experience below.
- Money Saving:** (1).It would be good result too to reuse the **used antibody in 1XBB**. Usually, you keep the used antibody (primary and second antibody together or separated) in **1XBB at 4° C for 3-4 weeks**. **Notice, to make sure the used 1 X BB** with antibody that were not any contamination and not any milk/BSA's deposit. (2).**1X BB** could increase the effective binding affinity between antigen and the antibody, so to make your antibody saving, see the Fig.2, A and B below.

Kit Content:

- Kit content table

Cat#	Name	Volume	Working for sheet	Storage at	Price(\$)
JZ-009	20xBinding Buffer(20xBB)	10 ml/bottle x2	10ml/sheet for 40-20; 8ml/each for 50-25;	5-29°C for 1 year from the open day	58.86/kit
	100x Binding Enhancer (100xBE)	4 ml/bottle x1			

- Below are not included in the kit, but, will be used in this protocol. To be provided by you self.

1). Milk/ BSA Powder*. 2).The acceptable Western Wash Buffer you used; **Or** to order **Western Blot Wash Buffer (JZ-011)**, which is better for a desired result with the clear background on your western blot.

- Make **1x Binding Buffer (1xBB)** to refer the table below**:

Total 1xBB making(ml)	10	20	30	40	50	60	70	80	90	100
20xBB(ml)	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
100xBE(ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Distilled Water (ml)	9.4	18.8	28.2	37.6	47.5	56.4	65.8	75.2	84.6	94
Milk / BSA(g)*	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5

* 5% Milk or BSA is as the final concentration in **1xBB**. Either milk or BSA is chosen to refer the manual of an antibody you will be used.

** 1). Follow the table to make the **1x BB**, make sure the Milk / BSA is dissolved completely. The ready **1xBB** could be kept at 4° C for 5-6 weeks, and which will work well too if it to be kept over 5-6 weeks without any contamination and /or any deposit of milk/BSA.

2). Also follow the table to make **1x BB** without milk/BSA, to be kept at RT for 3-6 month, at 4° C for 1 year with any contamination. Add milk/ BSA percent before using it.

Half Hour Protocol (For the primary & Second antibody to be used together):

- The ready Western membrane is blocked in wash buffer contained 5% Milk/BAS, to be shaking at RT for 2 minutes (5-10 min. are allowed).
- Put the blocked Membrane in the **1x BB** contained the acceptable primary and second anti-body together, then, to be shaking at RT for 20 minutes*.
- Rinse the membrane with wash buffer for 4-6 times and continue to wash it with wash buffer at RT, shaking for 2 minutes by 4 times.
- The membrane has been ready to be developed with ECL (**JZ-010**) for the Western Blot Result on the film.

One Hour Protocol (For the primary & Second antibody to be used separately):

- The ready Western membrane is blocked in wash buffer contained 5% Milk (or BAS), to be shaking at RT for 2-4 minutes (5-10 min. are allowed).
- Put the blocked Membrane in the **1xBB** contained acceptable primary antibody, to be shaking at RT for 20 minutes*.
- Rinse the membrane with wash buffer for 4-6 times and continue to be washed with wash buffer at RT, shaking for 2 minutes** by 4 times.
- Put the membrane in **1xBB** with acceptable second antibody; shaking at RT for 20 minutes.
- Rinse the membrane with wash buffer for 4-6 times and continue to wash it with wash buffer at RT, shaking for 2 minutes** by 4 times.
- The membrane has been ready to be developed with ECL (**JZ-010**) for the Western Blot Result on the film.

* 30-60 minutes are allowed if an antibody to be weaker expression; ** 5-10 are allowed if an antibody with a not desired background

Working Experience (data were gained with JZ-009 and JZ-011)

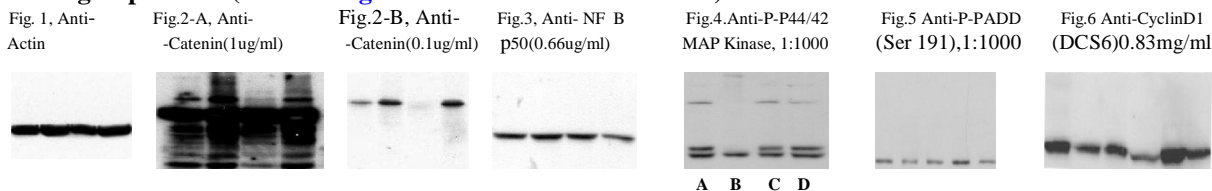


Fig.1. Mouse tissue protein, anti actin used 1: 30,000.

Fig.2. A and B; Mouse tissue protein, the antibody – -Catenin (eBioscience, cat#: 14-6765) were used in the high (Fig.2-A) and low (Fig.2-B) concentration after stripping (with Kit JZ-008)

Fig.3. Human cell line protein, antibody-NF B p50, E-10 (Santa Cruz, Cat#, sc-8414).

Fig. 4, Human cancer tissue (B, Cell line only) protein. The Phospho-P44/42 (thr202/tyr204); MAP Kinase antibody (# 9101, Cell Signal, 1:1000).

Fig. 5, Human cancer cell line protein, Anti-P-FADD (ser 191) (#2785, Cell Signal Tech.) 1:1000 used.

Fig. 6, the proteins were from the human cell line stably transfected with anti-Cyclin D1 (# RM-9104-SO, Lab. Vision Corporation.), 0.83 mg/ml.

Reference:

- Tibes R, et al.: Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. Mol Cancer Ther. 2006 Oct; 5(10):2512-21.
- Golding MC, et al.: Suppression of prion protein in livestock by RNA interference. Proc Natl Acad Sci U S A. 2006 Apr 4; 103(14):5285-90.
- Bleuming SA, et al.: Altered bone morphogenetic protein signalling in the Helicobacter pyloriinfectedstomach. J. Pathol, 2006 Jun; 7-9(2): 190-7.