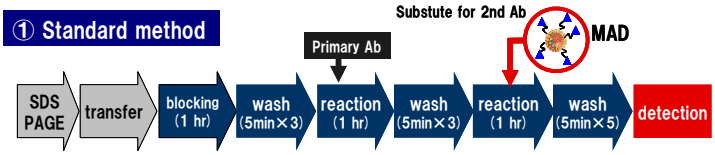
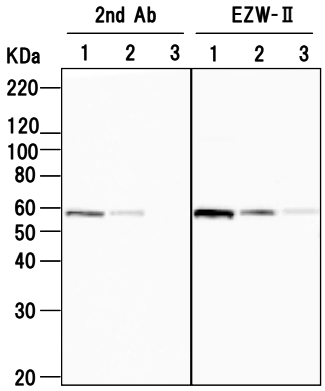
Experimantal Examples of Easy-WESTERN (EZW) -II

① Standard method



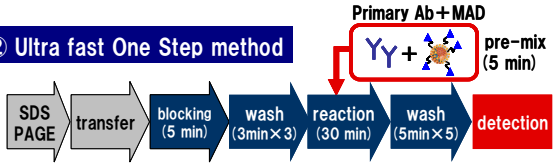
HuH7 Cell Lysate
primary ab : anti-vimentin, mouse IgG2a(1/5000)
2nd ab : anti-mouse IgG-HRP, rabbit poly (1/5000)
or MAD (1/2000)



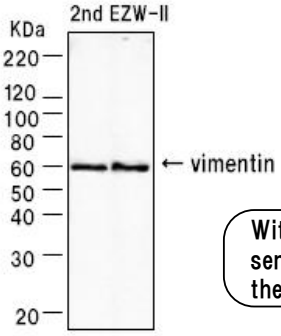
antigen
1:1/1
2:1/10 dilution
3:1/50 dilution

Strong signal even at low antigen level

② Ultra fast One Step method

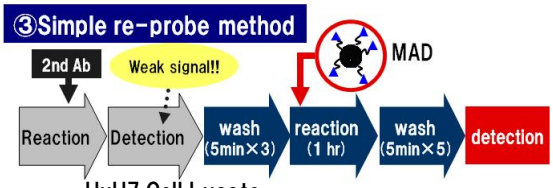


HuH7 Cell lysate
primary ab: anti-vimentin, mouse IgG2a (1/2500)
2nd ab: anti-mouse IgG-HRP 1/500 with ordinary pc. or MAD 1/2000 with ultra fast pc.

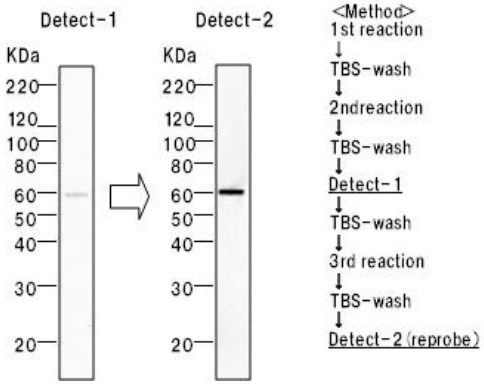


With total 60 min, equal sensitivity and background to the ordinary method achievable

③Simple re-probe method

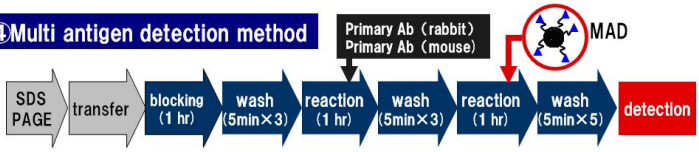


HuH7 Cell Lysate
primary ab :anti-vimentin, mouse IgG2a (1/1000)
2nd ab :anti-mouse IgG-HRP (rabbit poly, 1/5000)
reprobing : MAD(1/2000)

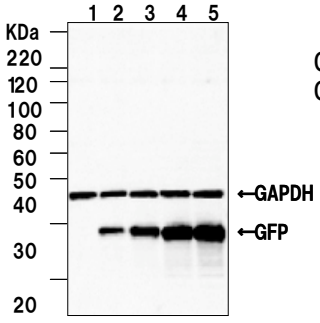


Simply add MAD reagent to membrane with weak signals to get strong signal

④Multi antigen detection method



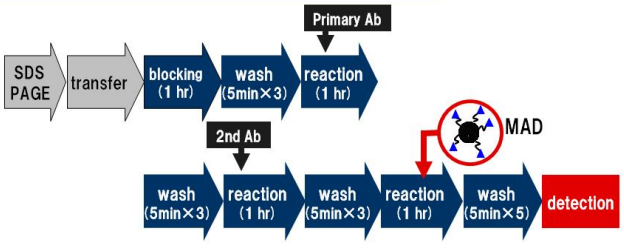
HuH7 Cell Lysate
primary ab: anti-GFP, Rabbit poly (1/2000)
anti-GAPDH, Rabbit mono (1/10000)
2nd ab.: MAD (1/2000)



GAPDH constant
GFP increases from 1 to 5

Simultaneous detection enables more accurate quantitative analysis of antigens.

⑤ Third reaction method for high sensitivity



The third reaction can be eliminated by pre-mixing of 2nd Ad and MAD reagent.

As the additional signal enhancer to expectedly weak signals

HuH7 Cell Lysate
primary ab):anti-vimentin, mouse IgG2a(1/1000)
2nd ab):anti-mouse IgG-HRP(rabbit poly (1/5000)
enhancing reaction : MAD(1/2000)