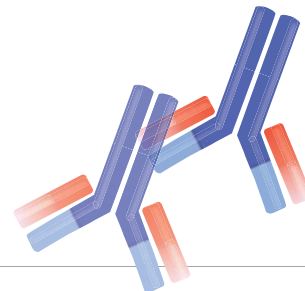


Epitope Tag Antibodies

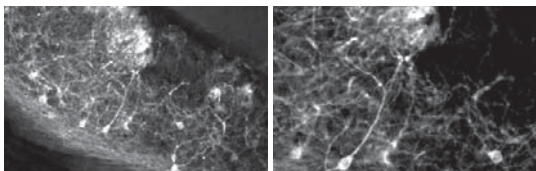


NACALAI TESQUE, INC. carries a family of epitope tag antibodies for the detection and purification of the recombinant proteins. Most of Nacalai's tag antibodies are highly specific mouse monoclonal antibodies.

• Anti-GFP (Rat IgG2a), Mono (GF090R)

Clone	: GF090R
Isotype	: IgG2a (Rat)
Product form	: Liquid
Immunogen	: His-GFP (full length) fusion protein
Application	: Immunohistochemistry 1:1000-1:2000
	: Western Blotting 1:1000-1:2000
	: ELISA 1:2000-1:20000

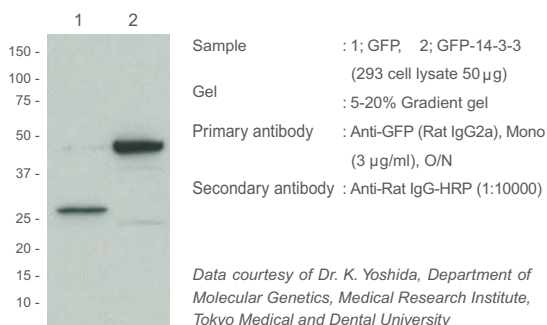
Immunohistochemistry



Sample	: Mouse brain (nerve cell)
Primary antibody	: Anti-GFP(Rat IgG2a), Mono (1:1000) RT, O/N
Secondary antibody	: Anti-Rat IgG-Cy3 (1:300) RT, 1 hr
Blocking	: 5% Normal goat serum/0.2% TritonX-100 in PBS
Fixing method	: 4% Paraformaldehyde

Data courtesy of Dr. Y. Yoshihara, RIKEN Brain Science Institute

Western Blotting



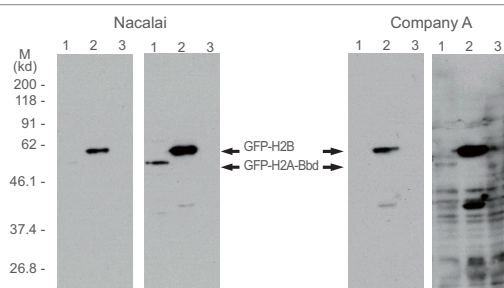
Reference

- Nakamura, M. *et al. Molecular Vision* **16**, 425-437 (2010)
- Nishide, K. *et al. PLoS ONE* **4**(8), e6869 (2009)
- Nagao, M. *et al. The Journal of Cell Biology* **183**(7), 1243-1257 (2008)
- Ono, K. *et al. Development Biology* **320**(2), 356-468 (2008)
- Nakashiba T. *et al. Science* **319**(5867), 1260-1264 (2008)
- Batista-Brito R *et al. The Journal of Neuroscience* **28**(15), 3966-3975 (2008)
- Esumi S. *et al. Neuroscience Research* **60**(4), 439-451 (2008)
- Fogarty M. *et al. The Journal of Neuroscience* **27**(41), 10935-10946 (2007)
- Sasamura T. *et al. Development* **134**, 1347-1356 (2007)
- Sato Y. *et al. The Journal of Neuroscience* **27**(7), 1606-1615 (2007)
- Yamada M. *et al. Stem Cells* **25**(3), 562-570 (2007)
- Togashi H. *et al. The Journal of Cell Biology* **174**, 141-151 (2006)
- Ogata M. *et al. Molecular and Cell Biology* **26**(24), 9220-9231 (2006)
- Sato Y. *et al. The Journal of Neuroscience* **25**(20), 4889-4897 (2005)

• Anti-GFP (Mouse IgG1-k), Mono (GF200)

Clone	: GF200
Isotype	: IgG1-k (Mouse)
Product form	: Liquid
Immunogen	: His-GFP (full length) fusion protein
Application	: Western Blotting 1:1000-1:2000
	: ELISA 1:2000-1:20000

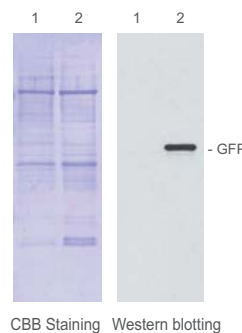
Western Blotting



[Exposure]	: 1 min 10 min 1 min 10 min
[Primary antibody]	: mouse monoclonal anti-GFP (3.6 µg/ml) rabbit polyclonal anti-GFP (1:1000)
[Secondary antibody]	: anti-mouse IgG (1:1000) anti-rabbit IgG (1:1000)
[Sample]	: 1. HeLa: GFP-H2A-Bbd 2. HeLa: GFP-H2B 3. HeLa

Data courtesy of Dr. H. Kimura, Horizontal Medical Research Organization, Kyoto University Faculty of Medicine

Western Blotting



Sample	: Protein extract from seedlings of Arabidopsis (10 mg) 1. Non-transformants (Control) 2. Transformants
Primary antibody	: Anti-GFP (Mouse IgG1-κ) (1:5000)
Secondary antibody	: Anti-Mouse IgG-HRP (1:2000)

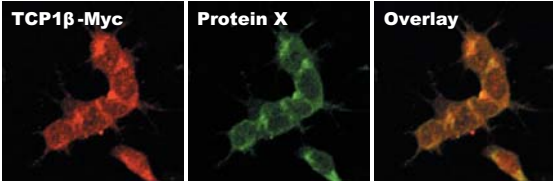
Data courtesy of Dr. T. Shimada, Department of Botany, Graduate School of Science, Kyoto University

Epitope Tag Antibody (continued)

● Anti-c-Myc (Mouse IgG1-k), Mono (MC045)

Clone	: MC045	
Isotype	: IgG1-k (Mouse)	
Product form	: Liquid	
Immunogen	: c-Myc synthetic peptide [EQKLISEEDL] conjugated with KLH	
Application	: Western Blotting	1:1000-1:2000
	: Immunoprecipitation	1:400-1:1000
	: Immunocytochemistry	1:400-1:1000
	: ELISA	1:2000-1:20000

Immunocytochemistry



TCP1β-Myc **Protein X** **Overlay**

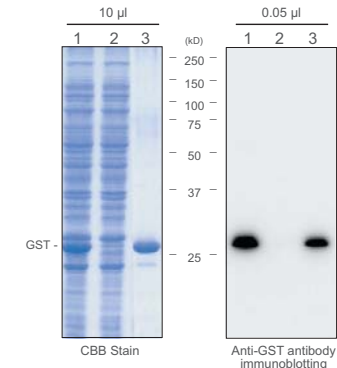
Sample : SH-SY5Y cell in which c-Myc-tagged TCP1β is expressed
 Primary antibody : Anti-c-Myc (Mouse IgG1-κ) (2.5 μg/ml)
 Anti-Protein X
 Secondary antibody : Anti-Fluor 546-conjugated antibody (1:400)
 Anti-Fluor 488-conjugated antibody (1:400)

Data courtesy of RIKEN Brain Science Institute

● Anti-GST (Mouse IgG2a-k), Mono (GS019)

Clone	: GS019	
Isotype	: IgG2a-k (Mouse)	
Product form	: Liquid	
Immunogen	: Glutathione-S-Transferase (GST)	
Application	: Western Blotting	1:1000-1:2000
	: Immunoprecipitation	1:400-1:1000
	: ELISA	1:2000-1:20000

Western Blotting



10 μl 0.05 μl

1 2 3 (kD) 1 2 3

250 -
150 -
100 -
75 -
50 -
37 -
25 -

GST -

CBB Stain Anti-GST antibody immunoblotting

Sample:
 1. Lysate from *E. coli* expressing GST protein
 2. Glutathione Sepharose flow-through fraction
 3. Glutathione Sepharose elution fraction


Gel : 12.5% SDS-PAGE
 Blocking : Blocking One (Nacalai Tesque) /1 hr at RT
 Primary antibody : Anti-GST (Mouse IgG2aκ) (1:2000 with Blocking One) /1 hr at RT
 Wash : TBS-T (5 min x 3)
 Secondary antibody: Anti-Mouse Ig-HRP (1:1000 with TBS-T)/30 min at RT
 Wash : TBS-T (5 min x 3)
 Detection : Luminol Reagent (Santa Cruz)

Data courtesy of Dr. Y. Shimizu, The Institute of Medical Science, The University of Tokyo

● Anti-6xHis (Mouse IgG1a-k), Mono (HI192)

Clone	: HI192	
Isotype	: IgG1-k (Mouse)	
Product form	: Liquid	
Immunogen	: 6xHis synthetic peptide [HHHHHHH] conjugated with KLH	
Application	: Western Blotting	1:1000-1:2000
	: ELISA	1:2000-1:20000

Western Blotting



Sample:
 Lysate from Sf9 cells in which His-tagged Drosophila PTEN is expressed

199 -
124 -
98 -
68 -
48 -

Filter : FluoroTrans [PALL]
 Blocking : 5% non-fat dry milk/PBS (30 min)
 Primary antibody : Anti-6xHis (Mouse IgG1-k), Monoclonal (HI192)
 Wash : 0.25% Tween-20/TBS (10 min x 3)
 Secondary antibody : Anti-Mouse IgG-HRP (1:1000) /2% BSA/0.25% Tween-20/TBS (30 min)
 Wash : 0.25% Tween-20/TBS (10 min x 5)
 Detection : Luminol Reagent (Santa Cruz)

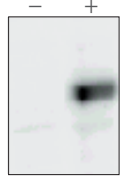
Data courtesy of Dr. A. Maehama, The Tokyo Metropolitan Institute of Medical Science (RINSHOKEN)

● Anti-V5 (Mouse IgG1-k), Mono (V5005)

Clone	: V5005	
Isotype	: IgG1-k (Mouse)	
Product form	: Liquid	
Immunogen	: V5 synthetic peptide [GKPIPPLLGLDST] conjugated with KLH	
Application	: Western Blotting	1:1000-1:2000
	: Immunoprecipitation	1:400-1:1000
	: ELISA	1:2000-1:20000

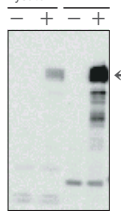
Western Blotting and Immunoprecipitation

(A) Western Blotting - VE-Cad. -V5



Sample : Lysate from 293T cell in which V5-tagged (C-terminal) pCDNA dest 40-VE cadherin is expressed
 Antibody : Anti-V5 (Mouse IgG1-κ) (1:5000)

(B) Immunoprecipitation - VE-Cad. -V5



lysate IP

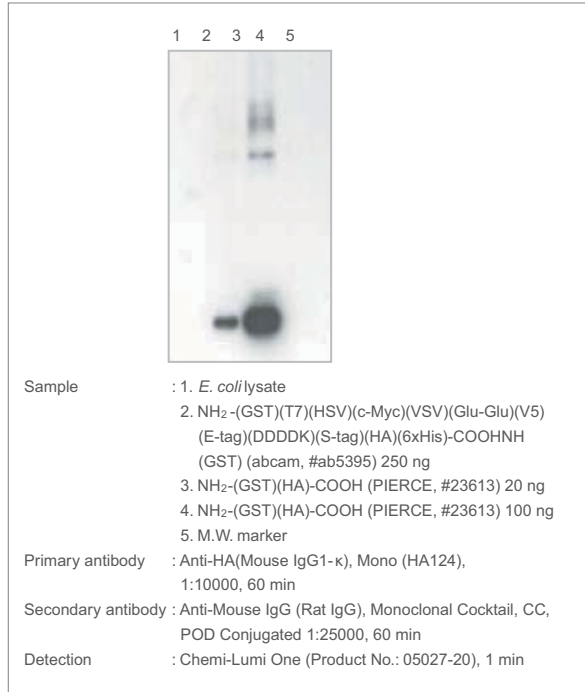
- + - +

Sample : Lysate (A)
 Antibody : Anti-V5 (Mouse IgG1-κ) (1 μl)
 Western Blotting: Anti-V5 (Mouse IgG1-κ) (1:2000)

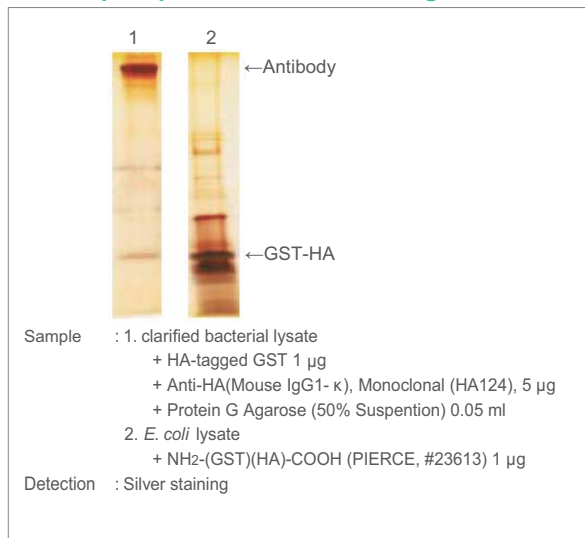
● Anti-HA (Mouse IgG1-κ), Mono (HA124)

Clone : HA124
 Isotype : IgG1-κ (Mouse)
 Product form : Liquid
 Immunogen : Glutathione-S-Transferase (GST)
 -HA[YPYDVPDYA-COOH] fusion protein
 Application : Western Blotting 1:10000-1:30000
 ELISA 1:2000-1:20000

Western Blotting



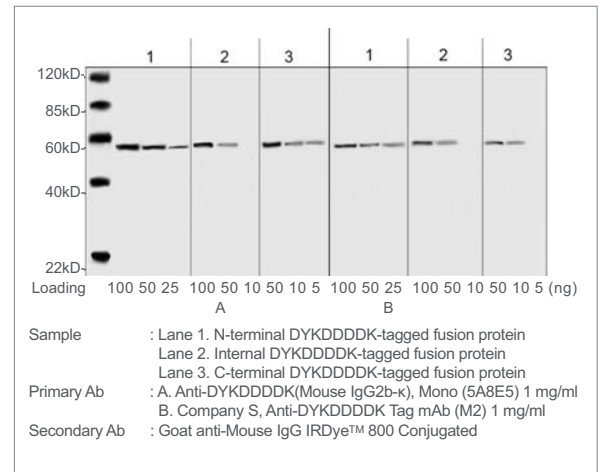
Immunoprecipitation and Gel staining



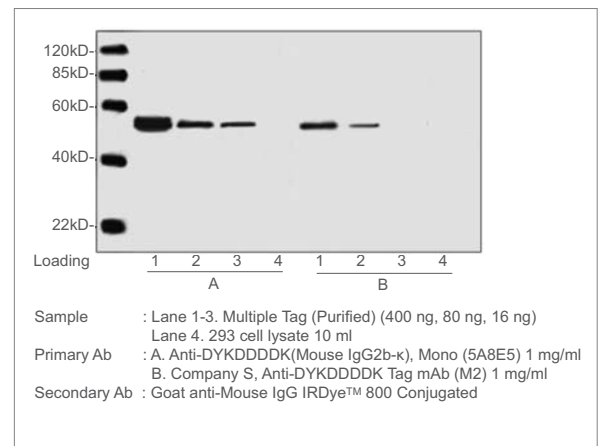
● Anti-DYKDDDDK(Mouse IgG2b-κ), Mono

Clone : 5A8E5
 Isotype : IgG2b-κ (Mouse)
 Product form : Lyophilized form
 Specificity : Anti-DYKDDDDK recognizes C-terminal, N-terminal
 and internal tagged fusion proteins.
 Concentration : 0.5 mg/ml, lyophilized with PBS, pH7.4, containing
 0.02% sodium azide.
 Immunogen : A synthetic peptide (DYKDDDDK) coupled KLH
 Application : Western Blotting 0.1-1.0 μg/ml
 Immunoprecipitation 1 μg/ml
 Immunofluorescent 1 μg/ml
 : ELISA 0.05-0.2 μg/ml

Western Blotting



Western Blotting



Epitope Tag Antibody (continued)

Ordering Information

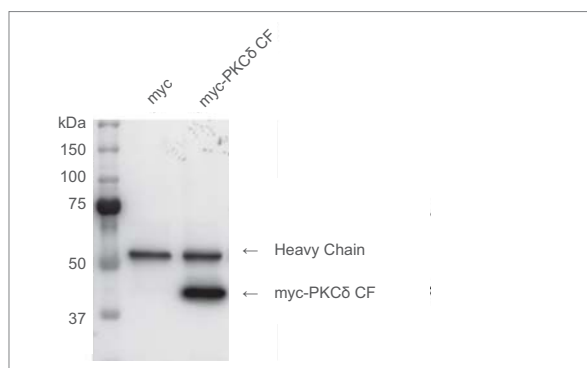
Product Name	Application	Storage	Product No.	PKG Size
Anti-c-Myc (Mouse IgG1-k), Monoclonal (MC045)	WB, IP, ICC, ELISA	R	04362-76	50 µg
			04362-34	200 µg
Anti-GFP (Mouse IgG1-k), Monoclonal (GF200)	WB, ELISA	R	04363-66	50 µg
			04363-24	200 µg
Anti-GFP (Rat IgG2a), Monoclonal (GF090R)	WB, IHC ELISA	R	04404-26	50 µg
			04404-84	200 µg
Anti-GST (Mouse IgG2a-k), Monoclonal (GS019)	WB, IP ELISA	R	04435-84	50 µg
			04435-26	200 µg
Anti-HA (Mouse IgG1-k), Monoclonal (HA124)	WB, ELISA	R	06340-96	50 µg
			06340-54	200 µg
Anti-6xHis (Mouse IgG1-k), Monoclonal (HI192)	WB, ELISA	R	04428-26	50 µg
			04428-84	200 µg
Anti-V5 (Mouse IgG1-k), Monoclonal (V5005)	WB, IP ELISA	R	04434-94	50 µg
			04434-36	200 µg
Anti-DYKDDDDK (Mouse IgG2b-k), Mono	WB, IP IF, ELISA	F	NU01102	200 µg

[Storage] R = Refrigerator, F = Freezer

Labeled Epitope Tag Antibody

• Anti-c-Myc, Agarose Conjugate

Western Blotting



Clone : MC045
 Isotype : IgG1a-κ (mouse)
 Size : 0.5 mg
 Antibody Protein : ca. 2 mg antibody / ml agarose
 Product form : 1:1 suspension in Dulbecco's PBS(-)

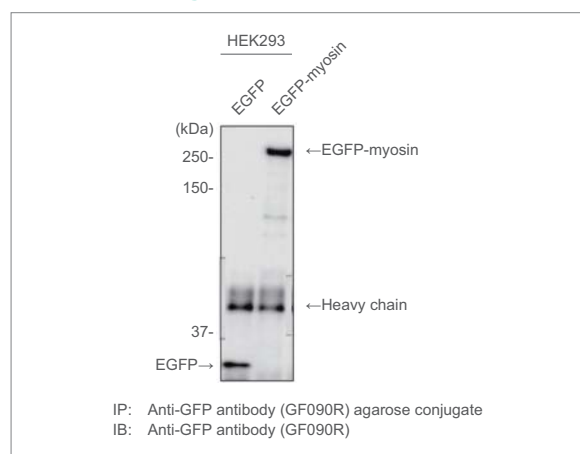
Cells transfected plasmid DNA including c-Myc Tag are lysed, and cell lysate was proceeded to immunoprecipitation with c-Myc-Agarose antibody. The figure above shows the result of wesetrn blotting analysis after immunoprecipitation.

Antibody
 Immunoprecipitate: Anti-c-Myc, Agarose conjugate (clone: MC045) (1:500)
 Western blotting: Anti-c-Myc (clone: MC045)

Data courtesy of a customer

• Anti-GFP (Rat IgG2a), Mono (GF090R), CC, Agarose Conjugate

Western Blotting



IP: Anti-GFP antibody (GF090R) agarose conjugate
 IB: Anti-GFP antibody (GF090R)

Samples: HEK-293 cell lysate expressing either EGFP or EGFP-Myosin (1 ml each)

Immunoprecipitation was performed with Anti-GFP agarose conjugate (20 ul) at 4°C for 2 hours.

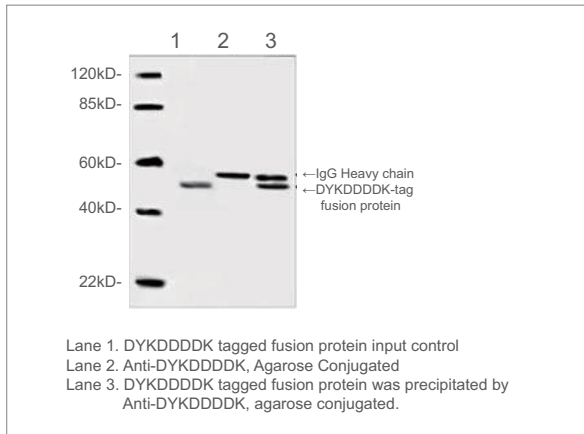
Data courtesy of Dr. A. Kakizuka, Laboratory of Functional Biology, Graduate School of Biostudies, Kyoto University

Referenes

Kumeta, M *et al.* Molecular mechanisms underlying nucleocytoplasmic shuttling of actinin-4. *J Cell Sci.* **123**(Pt 7):1020-30 (2010)

● Anti-DYKDDDDK (Mouse), Monoclonal Agarose Conjugate

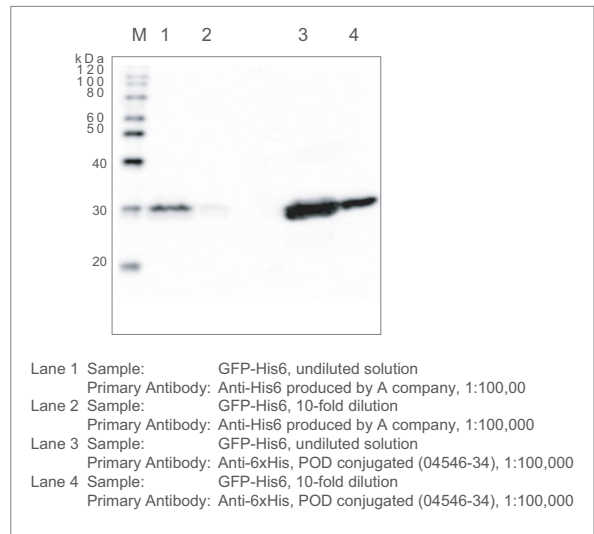
Western Blotting



Clone : 6H8E4
Degree of substitution : Approx. 8.5 mg Anti-DYKDDDDK mAb/ml settled resin
Capacity : > 1 mg/ml settled resin
Product form : 1x PBS containing 50% glycerol

● Anti-6xHis, POD Conjugated

Western Blotting



Clone : HI192
Isotype : IgG1a-κ (mouse)
Product form : Liquid
Immunogen : 6xHis synthetic peptide [HHHHHH]
: conjugated with KLH
Application : Western blot ting 1:1000 - 1:2000
: ELISA 1:30000 - 1:60000

Each concentration of GFP-His6 protein synthesized by in vitro translation were applied 2.5 μl to gel.

● Anti-c-Myc, POD Conjugated

Clone : MC045
Isotype : IgG1a-κ (mouse)
Product form : Liquid
Immunogen : c-Myc synthetic peptide [EQKLISEEDL]
conjugated with KLH
Application : Western blotting 1:1000 - 1:2000
ELISA 1:30000 - 1:60000

● Anti-V5, POD Conjugated

Clone : V5005
Isotype : IgG1-κ (mouse)
Product form : Liquid
Immunogen : V5 synthetic peptide [GKPIPNPLLDST]
conjugated with KLH
Application : Western blotting 1:1000 - 1:2000
ELISA 1:8000 - 1:16000

● Anti-GST, POD Conjugated

Clone : GS019
Isotype : IgG2a-κ (mouse)
Product form : Liquid
Immunogen : Glutathione-s-Transferase (GST)
Application : Western blotting 1:4000 - 1:8000
ELISA 1:30000 - 1:60000

● Anti-GFP, POD Conjugated

Clone : GF200
Isotype : IgG1-κ (mouse)
Product form : Liquid
Immunogen : His-GFP (full-length) fusion protein
Application : Western blotting 1:1000 - 1:2000
ELISA 1:2000 - 1:4000

Ordering Information

Product Name	Application	Storage	Product No.	PKG Size
Anti-c-Myc (Mouse IgG1-κ), Monoclonal (MC045), AS, Agarose Conjugate	IP	R	04145-55	500 μg
Anti-GFP (Rat IgG2a), Monoclonal(GF090R), CC, Agarose Conjugate	IP	R	06083-05	500 μg
Anti-GFP (Mouse IgG1-κ), Monoclonal (GF200), AS, POD Conjugated	WB, ELISA	R	05178-34	50 μg
Anti-DYKDDDDK (Mouse), Monoclonal Agarose Conjugate	IP	F	NU01103	1 ml
Anti-c-Myc (Mouse IgG1-κ), Monoclonal (MC045), AS, POD Conjugated	WB, ELISA	R	04554-24	50 μg
Anti-GST (Mouse IgG2a-κ), Monoclonal (GS019), AS, POD Conjugated	WB, ELISA	R	04559-74	50 μg
Anti-6xHis (Mouse IgG1-κ), Monoclonal (HI192), AS, POD Conjugated	WB, ELISA	R	04546-34	50 μg
Anti-V5 (Mouse IgG1-κ), Monoclonal (V5005), AS, POD Conjugated	WB, ELISA	R	04578-24	50 μg

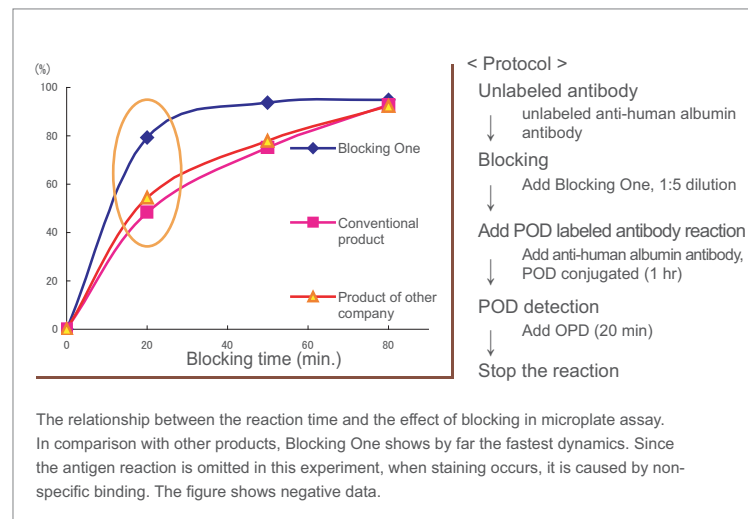
[Storage] R = Refrigerator, F = Freezer

Blocking One Series (High performance blocking reagents)

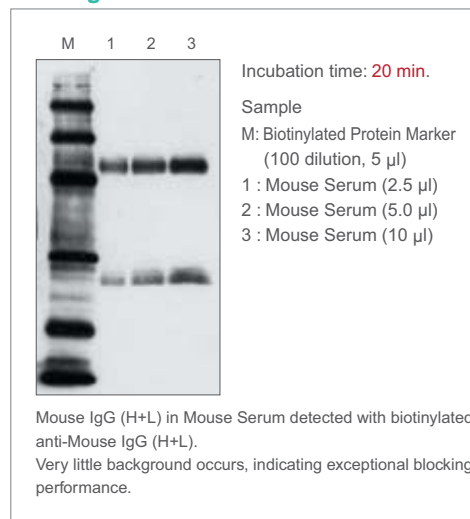
Blocking One

- » **Fast** In many assays a reduction of incubation time for blocking can be realized
- » **Easy-to-use** Simple storage in refrigerator even after opening the bottle

Comparison of Blocking Efficiency



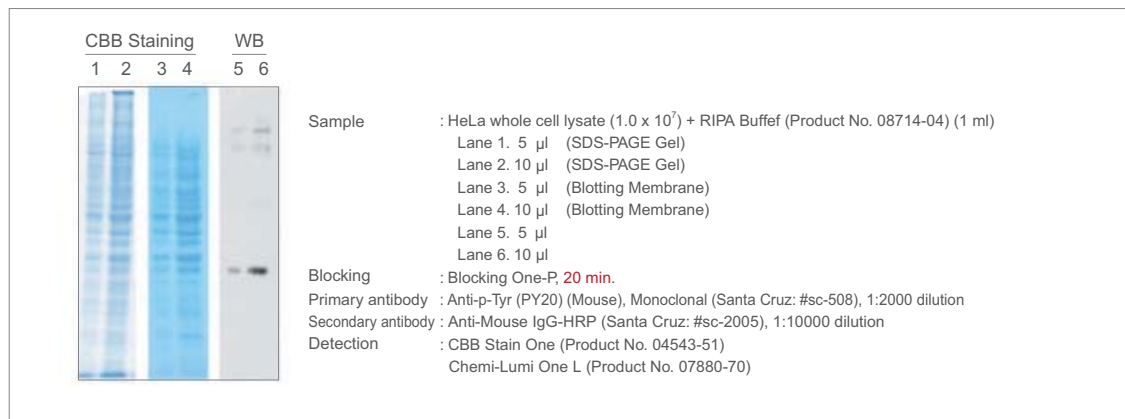
Detection of Mouse IgG(H+L) by Western Blotting



Blocking One-P

- » **Safe** Does not contain phosphorylated proteins
- » **Confirmed** Endogeneous phosphatase free
- » **High efficiency** More effective than 1% BSA

Western Blotting with Phospho-specific Antibody



Comparison

	Composition	Phospho-specific antibody applications	Blocking Efficacy
Blocking One-P	- High molecular weight compounds - BSA	+++	+++
Blocking One	- High molecular weight compounds - BSA - Casein	+	+++
Skim milk	- Casein	-	+
1% BSA	- BSA	++	+

Ordering Information

Product Name	Storage	Product No.	PKG Size
Blocking One	R	03953-95	500 ml
Blocking One-P	R	05999-84	200 ml

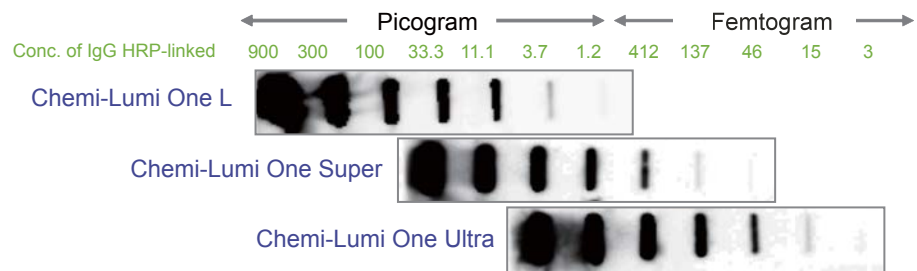
Chemi-Lumi One Series (High performance chemiluminescence substrates)

We offer three types of chemiluminescence detection kit as follows.

Selection Guide

Product Name	Chemi-Lumi One L	Chemi-Lumi One Super	Chemi-Lumi One Ultra
Product No.	07880	02230	11644
Usage	General Use	High Sensitivity	Ultrahigh Sensitivity
Lower Detection Limit	Low-picogram	Mid-femtogram	Low-femtogram
Required Working Solution	Approx. 0.125 ml / cm ²	Approx. 0.1 ml / cm ²	Approx. 0.1 ml / cm ²
Suggested Antibody Dilution	Primary: 1:1,000-1:5,000 Secondary: 1:20,000-1:100,000	Primary: 1:1,000-1:20,000 Secondary: 1:20,000-1:200,000	Primary: 1:5,000-1:100,000 Secondary: 1:100,000-1:500,000
Reaction Period	1 min.	1 min.	5 min.
Comparable to	ECL SuperSignal Pico	ECL Prime SuperSignal Dura	ECL Select SuperSignal Femto

Sensitivity



<Condition>

Antigen: Anti-Mouse IgG (Goat), HRP Conjugated (Santa Cruz, sc-2005)

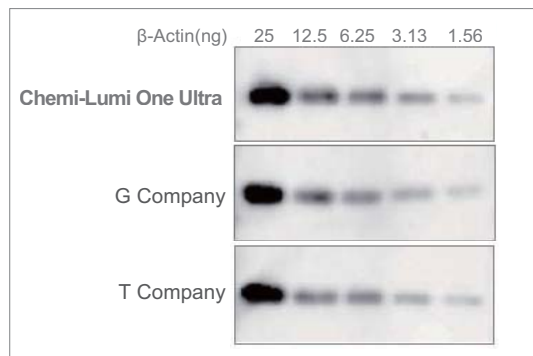
Detection: L (1 min.), Super (1 min.) and Ultra (5 min.)

Detector: LAS-3000 Super mode (Analyze 3 min. later after reaction with each substrate)

Expose time: 30 min.

Applications

1. [Chemi-Lumi One Ultra] Comparison data of sensitivity with competitors



<Condition>

Gel: 10% PAGE gel

Wash: 0.1% t-TBS(x1), pH7.4

Blocking: Blocking One

1st Ab: Anti-β-Actin Mouse Monoclonal Antibody, (Santa Cruz sc-47778), 1:300

2nd Ab: Anti-Mouse IgG (Goat), HRP Conjugated, (Santa Cruz sc-2005), 1:1,000

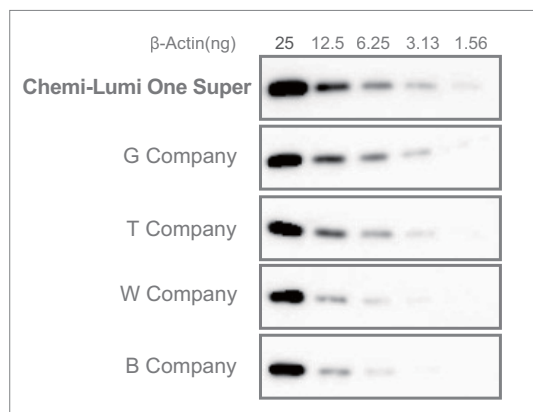
Detection period: 5 min.

Detector: LAS-3000 High mode

Expose time: 3 min.

Chemi-Lumi One Ultra shows equal to or higher sensitivity compared to competitors'.

2. [Chemi-Lumi One Super] Comparison data of sensitivity with competitors



<Condition>

Gel: 10% PAGE gel

Wash: 0.1% t-TBS, pH7.4

Blocking: Blocking One

1st Ab: Anti-β-Actin Mouse Monoclonal Antibody, (Santa Cruz sc-47778), 1:2,000

2nd Ab: Anti-Mouse IgG (Goat), HRP Conjugated, (Santa Cruz sc-2005), 1:30,000

Detection period: Chemi-Lumi One Super, **1 min.**

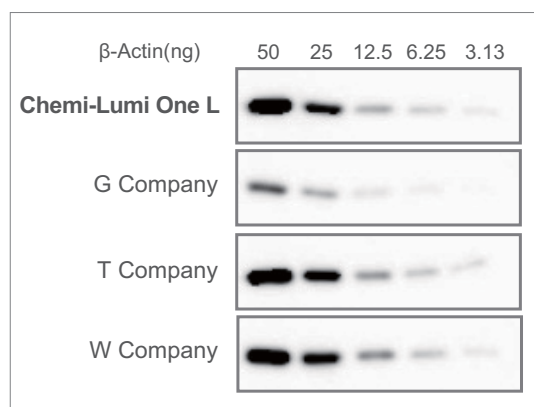
G, T, W, B Company 5 min.

Detector: LAS-3000 High mode

Expose time: 5 min.

Chemi-Lumi One Super is the most sensitive of competitors under the above condition, and its expose time is only 1 min., while others are required 5 min.

3. [Chemi-Lumi One L] Comparison data of sensitivity with competitors



<Condition>

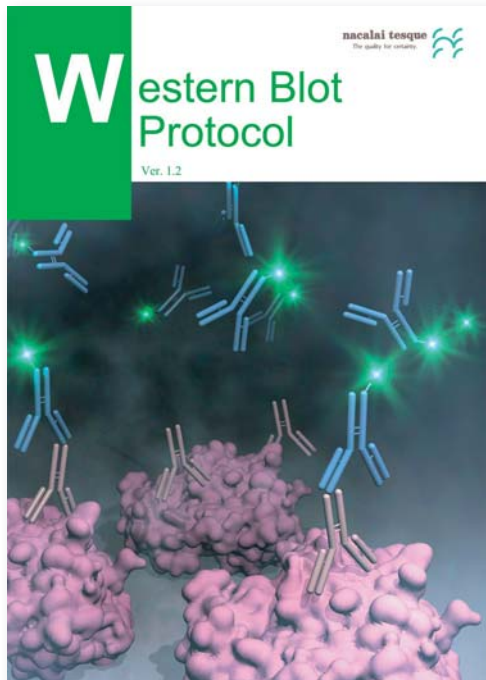
Gel: 10% PAGE gel
 Wash: 0.1% t-TBS(1x), pH7.4
 Blocking: Blocking One
 1st Ab: Anti- β -Actin Mouse Monoclonal Antibody, (Santa Cruz sc-47778), 1:1,000
 2nd Ab: Anti-Mouse IgG (Goat), HRP Conjugated, (Santa Cruz sc-2005), 1:20,000
 Detection period: Chemi-Lumi One L, 1 min.
 G, T, W Company 1min.
 Detector: LAS-3000 High mode
 Expose time: 5 min.

Chemi-Lumi One L offers the similar sensitivity as T and W company's products and higher sensitivity than G company's products.

Ordering Information

Product Name	Storage	Product Number	PKG Size
Chemi-Lumi One Ultra Luminol 50 ml, Peroxide 50 ml Sufficient substrate for 1,000 cm ² of blotting membrane	0-10°C	11644-40	1 kit
Chemi-Lumi One Super Luminol 50 ml, Peroxide 50 ml Sufficient substrate for 1,000 cm ² of blotting membrane	0-10°C	02230-30	1 kit
Chemi-Lumi One L Luminol 250 ml, Peroxide 250 ml Sufficient substrate for 4,000 cm ² of blotting membrane	0-10°C	07880-70	1 kit

Western Blot Protocol



Western Blot Protocol covering

- 1) Transfer
- 2) Confirming Transfer
- 3) Blocking
- 4) Detection
- 5) Western Blot Stripping

Please visit our website at www.nacalai.com to download PDF file or contact us to request a copy.

For research use only, not intended for diagnostic or drug use.

NACALAI TESQUE, INC.

Nijo Karasuma, Nakagyo-ku, Kyoto 604-0855 JAPAN

TEL : +81-(0)75-251-1730

FAX : +81-(0)75-251-1763

Website : www.nacalai.com

E-mail : info.intl@nacalai.com