

Epitope Tagging

Researchers use the process of epitope tagging to simplify the detection, characterization, and purification of proteins. First, a short amino acid epitope tag is introduced into a protein through recombinant DNA techniques. Subsequently, the functional, tagged protein can be detected and purified with one of our epitope-specific antibodies (Figure 31).

Epitope tagging eliminates the laborious, time-consuming task of producing an antibody to the specific protein being studied. Unlike a large fusion protein, these small epitope tags generally have minimal, if any, effects on the biological function of the tagged protein.

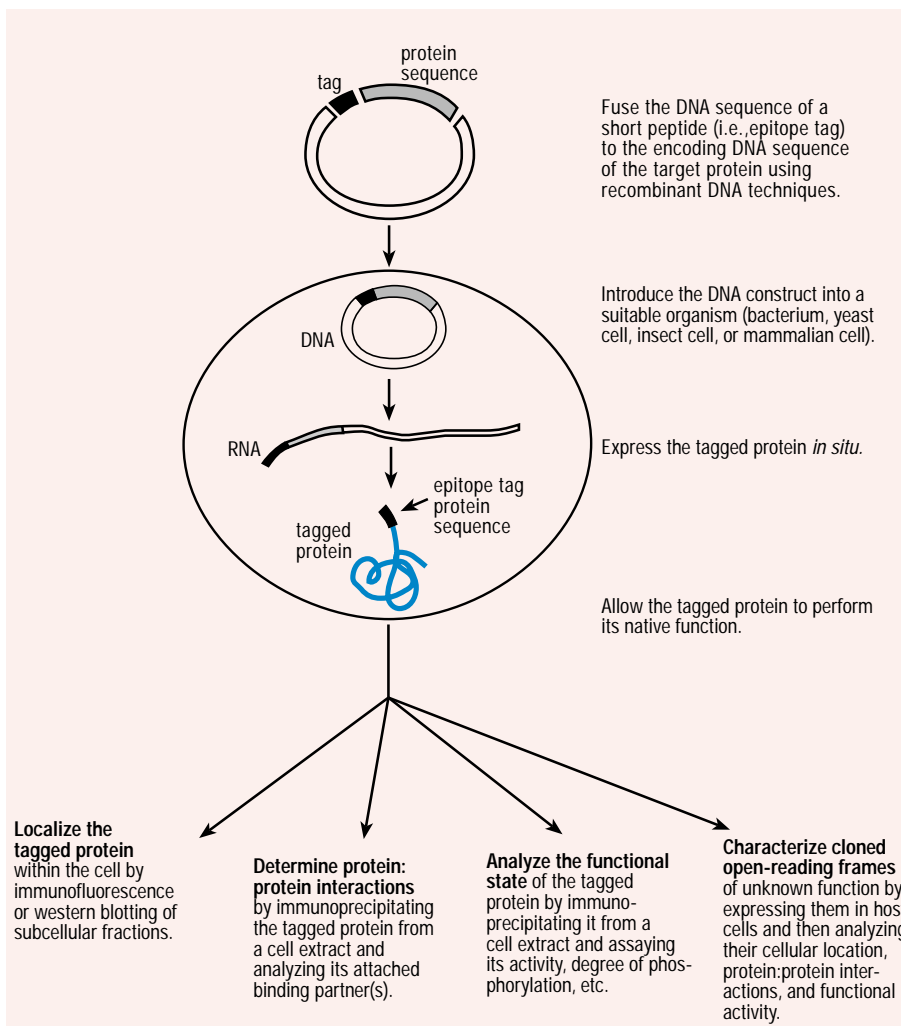


Figure 31. Epitope tagging principle and applications.

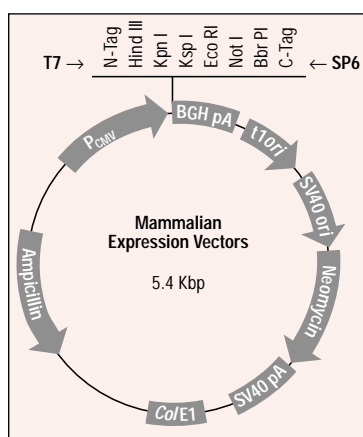


Figure 32. Map of the mammalian epitope-tagging expression vectors. Protein expression is enhanced by the efficient immediate-early human CMV promoter sequence. The vectors include a multiple cloning sequence with six different restriction enzyme cleavage sites for easy insertion of a DNA sequence in the desired reading frame. The different vectors provide options for both N- and C-terminal tagging of foreign protein with HA, VSV-G, and Protein C. His₆ labeling at the C-terminus is additionally provided in two of the vectors. A stop codon (TAA or TAG) is included at the end of the multiple cloning sequence to provide translation termination. For the three vectors lacking an N-terminal tag sequence (pMH, pMV, pMX), a Kozak sequence containing an initiator methionine start codon must be inserted during cloning to provide proper expression. Each vector can replicate episomally in cell lines expressing the SV40 large T antigen (e.g., COS-7) or containing the SV40 virus. SP6 and T7 RNA polymerase promoters are provided to allow synthesis of RNA probes.

Bacterial and Mammalian Expression Vectors for Epitope Tagging

For expression of epitope-tagged proteins in *E. coli* or mammalian cell lines

Description and use

Insert DNA of interest into one of six mammalian expression vectors for high-level transient and stable expression in a variety of mammalian cell lines (Table 15, Figure 32). Or choose from six new pUC-derived plasmids designed for efficient expression of epitope-tagged proteins in *E. coli* (Table 15, Figure 33). Each vector is provided as a 20 µg lyophilizate.

A control plasmid containing the appropriate tag (H, V, or X) fused on the N-terminus of β-galactosidase is also supplied free of charge with each corresponding vector; the H and V controls also contain the His₆ tag on the C-terminal.

Mammalian vector	Cat. No.	N-terminal tag	C-terminal tag
pHM6	1 814 664	HA	His ₆
pVM6	1 814 672	VSV-G	His ₆
pXM	1 814 699	Protein C	—
pMH	1 814 702	—	HA
pMV	1 814 729	—	VSV-G
pMX	1 814 737	—	Protein C

Bacterial vector	Cat. No.	N-terminal tag	C-terminal tag
pHB6	1 814 575	HA	His ₆
pVB6	1 814 583	VSV-G	His ₆
pXB	1 814 591	Protein C	—
pBH	1 814 605	—	HA
pBV	1 814 613	—	VSV-G
pBX	1 814 621	—	Protein C

Table 15. Bacterial and mammalian epitope-tagging expression vectors. Abbreviations:

B = bacterial vector, M = Mammalian vector, H = HA tag, X = Protein C tag, V = VSV-G tag, 6 = His₆ tag.

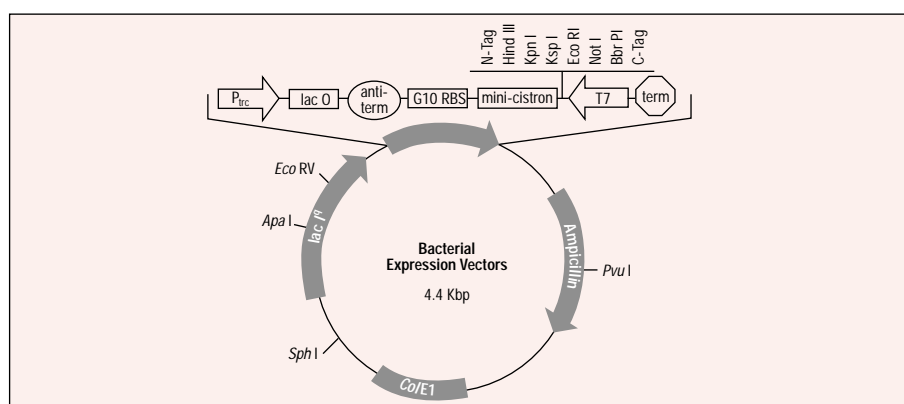


Figure 33. Map of the bacterial epitope-tagging expression vectors. High expression levels are driven by the combination *trp-lac* promoter. Addition of IPTG to the culture medium induces high-level expression of tagged protein. The vectors include ATG start codons and stop codons (TAA or TAG) flanking the multiple cloning sequence. The multiple cloning sequence provides six different restriction enzyme cleavage sites for easy insertion of a DNA sequence in the desired reading frame. The different vectors provide options for both N- and C-terminal tagging of foreign protein with HA, VSV-G, and Protein C. His₆ labeling at the C-terminus is additionally provided in two of the vectors. A T7 RNA polymerase promoter is provided for generation of anti-sense RNA probes.

Mammalian vectors

pHM6	<p>_____ HA _____ <i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ His₆ _____</p> <p>ATG TAC CCA TAC GAC GTC CCA GAC TAC GCT GGA AGC TTG GGT ACC TCC GCG GAG AAT TCG CGG CCG CTA CAC GTG CAT CAT CAT CAT CAT TAA</p> <p>Met Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Leu Gly Thr Ser Ala Glu Asn Ser Arg Pro Leu His Val His His His His His His ***</p>
pVM6	<p>_____ VSV-G _____ <i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ His₆ _____</p> <p>ATG TAC ACT GAT ATC GAA ATG AAC CGC CTG GGT AAG GAA GCT TGG GTA CCT CCG CGG AGA ATT CGC GGC CGC TAC ACG TGT CAT CAT CAT CAT CAT TAA</p> <p>Met Tyr Thr Asp Ile Glu Met Asn Arg Leu Gly Lys Glu Ala Trp Val Pro Pro Arg Arg Ile Arg Gly Arg Tyr Thr Cys His His His His His His ***</p>
pXM	<p>_____ Protein C _____ <i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI</p> <p>ATG GAA GAT CAG GTA GAT CCA CGG TTA ATC GAT GGT AAG AAG CTT GGG TAC CTC CGC GGA GAA TTC GCG GCC GCT ACA CGT GGG CCC TAT TCT ATA GTG TCA CCT AAA TGC TAG</p> <p>Met Glu Asp Gln Val Asp Pro Arg Leu Ile Asp Gly Lys Lys Leu Gly Tyr Leu Arg Gly Glu Phe Ala Ala Ala Thr Arg Gly Pro Tyr Ser Ile Val Ser Pro Lys Cys ***</p>
pMH	<p><i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ HA _____</p> <p>CCA AGC TTG GGT ACC TCC GCG GAG AAT TCG CGG CCG CTA CAC GTG TAC CCA TAC GAC GTC CCA GAC TAC GCT TAA</p> <p>Pro Ser Leu Gly Thr Ser Ala Glu Asn Ser Arg Pro Leu His Val Tyr Pro Tyr Asp Val Pro Asp Tyr Ala ***</p>
pMV	<p><i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ VSV-G _____</p> <p>CAA GCT TGG GTA CCT CCG CGG AGA ATT CGC GGC CGC TAC ACG TGC TAC ACT GAT ATC GAA ATG AAC CGC CTG GGT AAG TAA</p> <p>Gln Ala Trp Val Pro Pro Arg Arg Ile Arg Gly Arg Tyr Thr Cys Tyr Thr Asp Ile Glu Met Asn Arg Leu Gly Lys ***</p>
pMX	<p><i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ Protein C _____</p> <p>CCC AAG CTT GGG TAC CTC CGC GGA GAA TTC GCG GCC GCT ACA CGT GAA GAT CAG GTA GAT CCA CGG TTA ATC GAT GGT AAG TAA</p> <p>Pro Lys Leu Gly Tyr Leu Arg Gly Glu Phe Ala Ala Ala Thr Arg Glu Asp Gln Val Asp Pro Arg Leu Ile Asp Gly Lys ***</p>

Table 16. Sequences surrounding the multiple cloning region of the mammalian epitope-tagging expression vectors.

Bacterial vectors

pHB6	<p>_____ HA _____ <i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PII _____ His₆ _____</p> <p>ATG GGT TAC CCA TAC GAC GTC CCA GAC TAC GCT GGA AGC TTG GGT ACC TCC GCG GAG AAT TCG CGG CCG CTA CAC GTG CAT CAT CAT CAT CAT TAA</p> <p>Met Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Leu Gly Thr Ser Ala Glu Asn Ser Arg Pro Leu His Val His His His His His His ***</p>
pVB6	<p>_____ VSV-G _____ <i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ His₆ _____</p> <p>ATG GTA TAC ACT GAT ATC GAA ATG AAC CGC CTG GGT AAG GAA GCT TGG GTA CCT CCG CGG AGA ATT CGC GGC CGC TAC ACG TGT CAT CAT CAT CAT CAT TAA</p> <p>Met Val Tyr Thr Asp Ile Glu Met Asn Arg Leu Gly Lys Glu Ala Trp Val Pro Pro Arg Arg Ile Arg Gly Arg Tyr Thr Cys His His His His His His ***</p>
pXB	<p>_____ Protein C _____ <i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI</p> <p>ATG GAA GAT CAG GTA GAT CCA CGG TTA ATC GAT GGT AAG AAG CTT GGG TAC CTC CGC GGA GAA TTC GCG GCC GCT ACA CGT GTA AGG GAC TCC CTA TAG</p> <p>Met Glu Asp Gln Val Asp Pro Arg Leu Ile Asp Gly Lys Lys Leu Gly Tyr Leu Arg Gly Glu Phe Ala Ala Ala Thr Arg Val Arg Asp Ser Leu ***</p>
pBH	<p><i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ HA _____</p> <p>ATG GGA AGC TTG GGT ACC TCC GCG GAG AAT TCG CGG CCG CTA CAC GTG TAC CCA TAC GAC GTC CCA GAC TAC GCT TAA</p> <p>Met Gly Ser Leu Gly Thr Ser Ala Glu Asn Ser Arg Pro Leu His Val Tyr Pro Tyr Asp Val Pro Asp Tyr Ala ***</p>
pBV	<p><i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ VSV-G _____</p> <p>ATG GAA GCT TGG GTA CCT CCG CGG AGA ATT CGC GGC CGC TAC ACG TGC TAC ACT GAT ATC GAA ATG AAC CGC CTG GGT AAG TAA</p> <p>Met Glu Ala Trp Val Pro Pro Arg Arg Ile Arg Gly Arg Tyr Thr Cys Tyr Thr Asp Ile Glu Met Asn Arg Leu Gly Lys ***</p>
pBX	<p><i>Hind</i> III <i>Kpn</i> I <i>Ksp</i> I <i>Eco</i> RI <i>Not</i> I <i>Bbr</i> PI _____ Protein C _____</p> <p>ATG GGT AAG CTT GGG TAC CTC CGC GGA GAA TTC GCG GCC GCT ACA CGT GAA GAT CAG GTA GAT CCA CGG TTA ATC GAT GGT AAG TAA</p> <p>Met Gly Lys Leu Gly Tyr Leu Arg Gly Glu Phe Ala Ala Ala Thr Arg Glu Asp Gln Val Asp Pro Arg Leu Ile Asp Gly Lys ***</p>

Table 17. Sequences surrounding the multiple cloning region of the bacterial epitope-tagging expression vectors.

Application	Anti-HA (12CA5)	Anti-HA, High Affinity (3F10)
Western blot	+	++
Immuno-precipitation	+	+++
Immuno-fluorescence	+	++
Affinity chromatography	+	+/- ¹

Table 18. Comparison of applications of the two Anti-HA preparations.

¹ Elution of HA-tagged protein bound to the 3F10 antibody is only possible under denaturing conditions.

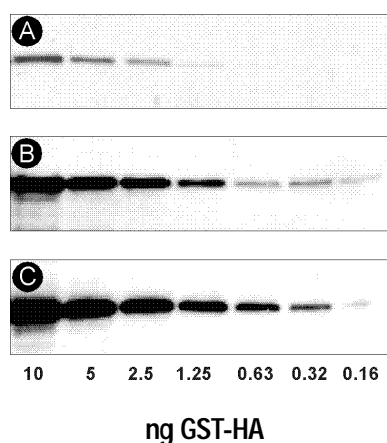


Figure 34. Comparative immunoblotting of an HA-tagged Glutathion-S-transferase with the Anti-HA (12CA5) and Anti-HA, High Affinity (3F10). HA-tagged Glutathion-S-transferase (GST-HA) was serially diluted to the indicated amounts, separated by SDS-PAGE and Western blotted. The primary Anti-HA antibodies were used at concentrations of 1 µg/ml (clone 12CA5) and 0.1 µg/ml (clone 3F10) respectively. Bound antibodies were detected using Anti-Mouse-peroxidase (12CA5, **A**), Anti-rat-peroxidase (3F10, **B**) and an Anti-rat-biotin/streptavidin-peroxidase (3F10, **C**).

Note that approximately 20-fold less GST-HA can be detected with a 10-fold lower antibody concentration using the high affinity 3F10 clone.

Antibodies to Epitope Tags

Detect and characterize proteins tagged with HA, Protein C, c-myc, VSV-G, His₆, or GFP

Roche Molecular Biochemicals offers antibodies, numerous antibody conjugates, and several related peptides and markers for the study of six different epitope tags.

■ Choose from a variety of tags

Introduce the HA or Protein C epitopes to tag proteins (see above for fusion vectors) for subsequent detection with Anti-HA or Anti-Protein C; these two tags are the preferred choices for most experiments. Study expression of two or more proteins simultaneously by detecting one protein tagged with the HA or Protein C epitopes and another tagged with the c-myc or VSV-G epitopes.

■ Choose from two separate Anti-HA preparations

Take either the original Anti-HA (clone 12CA5) or Anti-HA, High Affinity (clone 3F10), depending on your application (Table 18). For experiments in which sensitivity is not critical, use Anti-HA clone 12CA5. For higher sensitivity detection in Western blotting at 10-fold lower concentration (Figure 34) use the new 3FA Anti-HA. The 3F10 Anti-HA, High Affinity, preparation is especially useful for quantitative immunoprecipitation at 10- to 20-fold lower antibody concentrations than clone 12CA5 (Figure 34). Also, because clone 3F10 is a rat monoclonal antibody, it can be used with a rat secondary detection system to detect a HA-tagged protein while simultaneously using a mouse antibody and a mouse secondary detection system to identify a different epitope-tagged protein in the same sample.

■ Save steps with peroxidase conjugates of antibodies

Achieve sensitive Western blotting results after fewer handling steps and in less time by performing direct detection with peroxidase conjugates (Figure 35).

■ Localize proteins with fluorescent Anti-HA conjugates

Visualize tagged proteins within cells with fluorescein- or rhodamine-labeled Anti-HA antibodies (clone 12CA5). Use either antibody in combination with other markers to perform double labelling experiments.

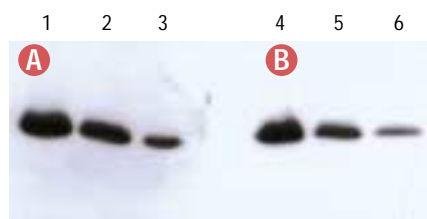


Figure 35. Sensitivity of direct and indirect methods for detecting HA-tagged protein in Western blotting. HA-GFP was expressed in *E. coli* and purified from cell extracts. Purified protein was analyzed on a Western blot using direct detection with Anti-HA-peroxidase (12CA5; Panel A) or indirect detection with Anti-HA (12CA5) and a secondary antibody (Panel B). The overall sensitivity (signal to noise) of the two detection methods is equivalent.

Lanes 1,4: 6 ng HA-GFP

Lanes 2,5: 3 ng HA-GFP

Lanes 3,6: 1 ng HA-GFP

■ Gently elute Protein C tagged fusion proteins from affinity columns

Anti Protein-C clone HPC-4 recognizes a 12 amino acid epitope (EDQVDPRLIDGK) of human Protein C. This sequence can be tagged to any gene of interest (for tagging vectors see above). Because HPC-4 binds only in the presence of calcium, the antibody is excellent for affinity purification of Protein C tagged fusion proteins combined with gentle non-denaturing elution. A major advantage of this system is that only one antibody is required to detect the recognized epitope regardless of its location in the tagged protein.

■ Use His₆ as an epitope tag

Detect existing His₆ fusion clones with this new Anti-His₆ antibody, which eliminates the need to reclone the target gene into different epitope tagging vectors (Figure 36).



Figure 36. Detection of His₆ fusion clones

with Anti-His₆. Various amounts of a GST-His₆ fusion protein were electrophoresed, Western blotted, and detected with Anti-His₆.

Lane 1: Multi-Tag Marker

Lane 2: 20 ng

Lane 3: 10 ng

Lane 4: 5 ng

Lane 5: 2.5 ng

Lane 6: 1.25 ng

■ Detect GFP fusion proteins

Choose our new Anti-GFP antibody preparation for detection of proteins fused with green fluorescent protein (GFP) in Western blot experiments or in immunoprecipitation applications. The antibody recognizes wild-type and mutant GFPs (Figure 37).

■ Simplify the sizing of epitope-tagged and fusion proteins

Simultaneously detect your tagged protein of interest and the Multi-Tag Marker using the same antibody. Use this mixture of six purified tagged-proteins – each labeled with glutathione-S-transferase (GST) and the HA, c-myc, or His₆ epitopes – to accurately size proteins on Western blots (Figure 38).

■ Use HA, c-myc, or VSV-G peptides for competitive elution of purified proteins

Elute epitope tagged proteins from affinity columns using either of three competitive epitope tag peptides.

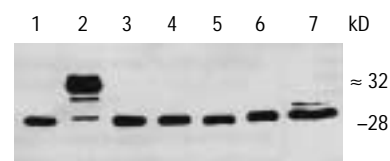


Figure 37. Western blot detection of GFP variants with anti-GFP (0.4 µg/µl).

Lane 1: Pure wild-type GFP

Lane 2: *E. coli* expressing (His)₆-HA-GFP

Lane 3: *E. coli* expressing M1-GFP

Lane 4: *E. coli* expressing M2-GFP

Lane 5: *E. coli* expressing M3-GFP

Lane 6: *E. coli* expressing Cycle 3-GFP

Lane 7: COS-1 lysate expressing Green Lantern GFP

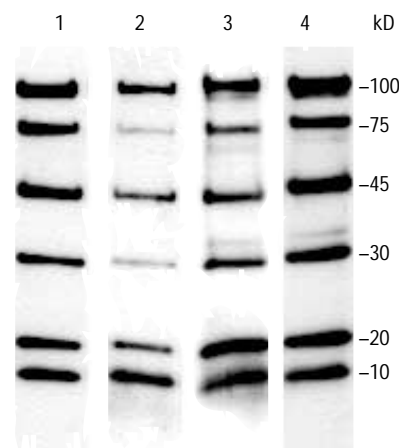


Figure 38. Use of the Multi-Tag Marker to size proteins on a Western blot. In each lane, the Multi-Tag Marker was detected with a different antibody to an epitope tag.

Lane 1: Anti-GST

Lane 2: Anti-HA

Lane 3: Anti-c-myc

Lane 4: Anti-His₆

Product	Cat. No.	Pack Size	Clone (Source)	Use
Anti-HA	1 583 816 1 666 606	200 µg 5 mg (1 ml)	12CA5 (mouse)	Use with a secondary detection system to detect and characterize proteins by Western blotting, and immunocytochemistry.
Anti-HA-biotin	1 666 851	100 µg (500 µl)	12CA5 (mouse)	Perform Western blots or immunocytochemistry with tagged proteins using an avidin-based detection system.
Anti-HA-fluorescein	1 666 878	100 µg (500 µl)	12CA5 (mouse)	Localize proteins in cell samples by immunofluorescence.
Anti-HA-peroxidase	1 667 475	50 µg (500 µl)	12CA5 (mouse)	Perform single-step detection on Western blots and immunocytochemistry.
Anti-HA-rhodamine	1 666 959	100 µg (500 µl)	12CA5 (mouse)	Localize proteins in cell samples by immunofluorescence.
Anti-HA, High Affinity	1 867 423 1 867 431	50 µg 500 µg	3F10 (rat)	Use with a secondary detection system to analyze proteins in applications requiring a high-affinity Anti-HA preparation (e.g., immunoprecipitation, Western blots, immunocytochemistry).
Anti-Protein C	1 814 598 1 814 516	200 µg 5 mg	HPC-4 (mouse)	Use with a secondary detection system to detect and characterize proteins by Western blotting, etc.
Anti-GFP	1 814 460	200 µg	mixture of clones 7.1 and 13.1 (mouse)	Use with a secondary detection system in Western blotting to confirm that transfected cells are producing GFP fusion proteins; immunoprecipitate GFP fusion proteins.
Anti-c-myc	1 667 149 1 667 203	200 µg 5 mg (1 ml)	9E10 (mouse)	Use with a secondary detection system to detect and characterize proteins by Western blotting, etc.
Anti-c-myc-peroxidase	1 814 150	500 µg (500 µl)	9E10 (mouse)	Perform single-step detection on Western blots.
Anti-VSV-G	1 667 351 1 667 360	200 µg 5 mg (1 ml)	P5D4 (mouse)	Use with a secondary detection system to detect and characterize proteins by Western blotting, etc.
Anti-VSV-G-peroxidase	1 814 133	500 µg (500 µl)	P5D4 (mouse)	Perform single-step detection on Western blots.
Anti-His ₆	1 922 416	100 µg	BMG-His-1 (mouse)	Use with a secondary detection system to detect and characterize proteins by Western blotting, etc.
Peptides and Molecular Weight Markers				
c-myc Peptide	1 667 246	5 mg	———	Free purified c-myc-tagged proteins by competitive elution.
HA Peptide	1 666 975	5 mg	———	Free purified HA-tagged proteins by competitive elution.
VSV-G Peptide	1 667 432	5 mg	———	Free purified VSV-G-tagged proteins by competitive elution.
rGFP	1 814 524	50 µg	———	Use as an experimental control to validate spectrophotometric and immunochemical detection methods.
Multi-Tag Marker	1 828 649	250 µl	———	Use the same antibody to detect both the protein of interest and this mixture of epitope-tagged protein molecular weight markers (tagged with HA, c-myc, His ₆ , and GST) on Western blots.