

For life science research only.
Not for use in diagnostic procedures.



Anti-Protein C Affinity Matrix

 **Version 09**

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Mouse monoclonal antibody (Clone HPC4), immobilized

Cat. No. 11 815 024 001 1 ml settled resin volume

Store the matrix at +2 to +8°C

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1. What this Product Does

Antibody type	Clone HPC4, mouse IgG ₁ κ
Formulation	<ul style="list-style-type: none">• The antibody is covalently coupled to the matrix (agarose beads) and is supplied as a 2-ml slurry containing 1 ml of beads in 1 ml of buffer (20 mM Tris, 0.1 M NaCl, 1 mM CaCl₂, and 0.09% sodium azide as preservative).• 4 mg of antibody is reacted per 1 ml of beads in the coupling reaction.• Includes plastic column with top and bottom caps.
Storage and Stability	<ul style="list-style-type: none">• Anti-Protein C Affinity Matrix is stable at +2 to +8°C through the expiration date printed on the label (24 months from date of manufacture). Do not freeze.• Anti-Protein C Affinity Matrix is shipped at ambient temperature.
Application	<p>Anti-Protein C Affinity Matrix is suitable for:</p> <ul style="list-style-type: none">• Affinity purification of Protein C-tagged proteins from crude protein extracts.• Immunoprecipitation of Protein C-tagged proteins. <p>Following purification, the tagged protein of interest may be analyzed by:</p> <ul style="list-style-type: none">• Western Blotting using the anti-Protein C antibody.• Silver Staining (or similar protein stain).
Specificity	Anti-Protein C recognizes the 12-amino acid sequence EDQVDPRLIDGK, which encodes residues 6 through 17 of the heavy chain of Protein C. In the presence of Ca ²⁺ , the antibody binds with high affinity and specificity to this sequence in native human protein C or in proteins tagged with this epitope. This efficient binding within the recombinant fusion protein occurs regardless of the site of incorporation of the epitope tag (<i>i.e.</i> , N terminus, C terminus, or within the reading frame).

2. How to Use this Product

2.1 Before you begin

Additional required equipment

- Rack or stand suitable for gravity collection of samples.
- 23G needle for adjustment of flow rate.
- Ⓢ This needle size is appropriate for 0.5 - to 1.0 - ml column bed volumes, but may be adjusted as needed for larger or smaller columns.

Additional required reagents

Prepare approximately 100 ml of the following reagents before beginning the affinity purification.

Buffer	Concentration
Equilibration buffer	20 mM Tris, pH 7.5; 0.1 M NaCl; 1 mM CaCl ₂ CaCl ₂ stock solution: 1 M CaCl ₂
Wash buffer	20 mM Tris, pH 7.5; 1.0 M NaCl; 1 mM CaCl ₂
EDTA elution buffer	20 mM Tris, pH 7.5; 0.1 M NaCl; 5 mM EDTA
Peptide elution buffer (optional procedure)	20 mM Tris, pH 7.5; 0.1 M NaCl; 1 mM CaCl ₂ ; 0.5 mg/ml synthetic protein C peptide
Column storage buffer	20 mM Tris, pH 7.5; 0.1 M NaCl; 1 mM CaCl ₂ ; 0.09% sodium azide
Regeneration buffer (optional procedure)	0.1 M glycine, pH 2.0

Important notes regarding sample preparation

Prepare protein extracts containing the Protein C-tagged protein of interest using any of a variety of standard methods (13). The following lysis buffers have performed well:

- **Bacterial extracts:** 20 mM Tris, pH 8.0; 100 mM NaCl; 1 mM CaCl₂; Complete Protease Inhibitor Cocktail Tablets, EDTA-free*
- **Mammalian extracts:** 50 mM Tris, pH 7.5; 150 mM NaCl; 0.1% Nonidet P40; 0.05% Deoxycholate, 1 mM CaCl₂; Complete Protease Inhibitor Cocktail Tablets, EDTA-free*
- Ⓢ Other cell lysis buffers may be more appropriate for individual applications. Avoid phosphate buffers to prevent precipitation.


To obtain optimal performance of the affinity column:

- Remove insoluble particulates from the protein extract prior to loading on the column. If insoluble particulates are present, remove particulates by centrifugation or by filtration.
- Add CaCl_2 stock solution to protein extract solution to obtain a final concentration of 1 mM Ca^{2+} .
- Use protease inhibitors to reduce proteolytic activity. Use Complete Protease Inhibitor Cocktail Tables, EDTA-free for most applications. Avoid EDTA, which inhibits antibody binding.
- Limit detergents to the lowest possible concentration levels necessary to obtain adequate cell lysis.
- Avoid overloading the column, which could result in decreased purity of final product.

** available from Roche Diagnostics*

2.2 Affinity purification

Column preparation

- 1 Attach lower cap to bottom of column, then place column on rack or stand above desired collection tube.
- 2 Gently invert anti-Protein C Affinity Matrix several times to thoroughly resuspend beads.
- 3 Pipet desired volume of slurry into column (suggested final settled bead volume is 0.5–1.0 ml).
- 4 Replace lower cap with 23G needle.
- 5 Drain Column storage buffer into collection tube. Adjust column flow rate to approximately 0.3–0.5 ml/minute. If flow rate is faster, adjust with a smaller gauge needle at this time.
 Do not allow column to dry out.
- 6 Immediately add 10 bed-volumes of Equilibration buffer (e.g., 10 ml for a 1-ml column), and allow buffer to drip through column.
- 7 Carefully remove needle and replace lower cap when buffer level reaches the top of the matrix bed.

Loading column

- 1 Determine amount of crude protein extract to be purified. Optimal starting volume is 1 – 5 ml for a 1-ml column. Optimal total protein concentration of extract is dependent upon expression levels of tagged protein. Use the following initial concentration:

If starting lysate is	then total protein loaded/ml of matrix should be
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bacterial	1-3 mg
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mammalian	3-6 mg
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- Ⓢ Column may become clogged if excessive total protein is loaded, or if insoluble materials have not been adequately removed from extract. Total protein loaded may be gradually increased with successive column runs; however, column should be able to flow at a rate of 0.1 ml/min or greater.

- 2 Add CaCl_2 from 1 M stock solution to protein extract in order to obtain a final concentration of 1 mM CaCl_2 .

Ⓢ Keep extract cold (+2 to +8°C).

- 3 Load protein extract on column and cap column tightly.

- 4 Invert column to mix. Place column on end-over-end rocker at +2 to +8°C. Incubate for a minimum of one hour, but not longer than overnight.

Column elution with EDTA

- 1 Allow column matrix to settle for 10 minutes after mixing. Remove top cap and replace lower cap with needle.

- 2 Collect lysate flow-through in a clean collection tube. Store this fraction at +2 to +8°C.

- 3 Wash column with a minimum of 10 bed-volumes of Wash buffer at +15 to +25°C to remove nonspecifically bound protein.

Ⓢ An OD_{280} reading performed at the end of this step will verify the final wash fractions contain no protein and are close to baseline levels (Wash buffer alone).

- 4 Re-equilibrate column with 10 bed-volumes of Equilibration buffer, and allow buffer to drain through column.

- 5 Immediately replace needle with lower cap and add 1 ml of EDTA Elution buffer/ml of column matrix. Incubate for 30 minutes at +15 to +25°C without shaking.

- 6 Replace needle and collect the first 1 ml elution fraction in a clean microfuge or equivalent collection tub.

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- 7 Repeat Steps 5 and 6 four more times with a 5 min incubation each time.
 - Ⓢ Column may be eluted with one 5-ml bed-volume of Elution buffer to save time, however, slightly lower yields of purified protein may result.
 - 8 Read OD₂₈₀ for each fraction and pool as desired. In most instances, the first three fractions will contain the bulk of the eluted protein.
 - 9 Keep samples cold (+2 to +8°C) until analysis.
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Column elution with peptide

-
- 1 Allow column matrix to settle for 10 minutes after mixing. Remove top cap and replace lower cap with needle.
 - 2 Collect lysate flow-through in a clean collection tube. Store this fraction at +2 to +8°C.
 - 3 Wash column with a minimum of 10 bed-volumes of Wash buffer at +15 to +25°C to remove nonspecifically bound protein.
 - Ⓢ An OD₂₈₀ reading performed at the end of this step will verify the final wash fractions contain no protein and are close to baseline levels (Wash buffer alone).
 - 4 Reequilibrate column with 10 bed-volumes of Equilibration buffer, and allow buffer to drain through column.
 - 5 Immediately replace needle with lower cap and add one bed volume of Peptide elution buffer (peptide concentration of 0.5 mg/ml). Incubate for 30 min at +15 to +25°C. Gently mix the matrix once or twice during the incubation step to increase recovery.
 - 6 Replace needle and collect the first 1 ml elution fraction in a clean microfuge or equivalent collection tub.
 - 7 Repeat Steps 5 and 6 four more times with a 5 minute incubation each time.
 - 8 Read OD₂₈₀ for each fraction and pool as desired. In most instances, the first three fractions will contain the bulk of the eluted protein.
 - 9 Keep samples cold (+2 to +8°C) until analysis.
-

Column reuse, regeneration, and storage

For many EDTA elution protocols (if the protein to be purified is obtained from the same cell extract), it is not necessary to regenerate the column. Simply:

- Wash with 10 bed-volumes of Wash buffer.
- Immediately reequilibrate with 10 bed-volumes of Equilibration buffer.
- Store tightly capped at +2 to +8°C in 2 bed-volumes of Column storage buffer.
- Ⓢ If different proteins are to be purified using the same column, the column must be stripped and regenerated between each purification. With peptide elution it is always necessary to regenerate the column:
- Strip column by running 20–30 bed-volumes of Regeneration buffer through the column.
- Immediately reequilibrate column with 10 bed-volumes of Equilibration buffer.
- Store tightly capped at +2 to +8°C in 2 bed-volumes of Column storage buffer.
- Ⓢ Other buffers and preservatives may be substituted, but have not been tested. Since calcium is a requirement for antibody binding with this system, avoid buffers containing phosphate to prevent precipitation.

Yield of purified protein

Yield of purified protein is dependent upon expression levels within crude extract. Typical results from a 1-ml column range from 2–10 nmol of purified protein. Using a whole-cell bacterial extract, which expressed Protein C-tagged β -gal, the column capacity was determined to be approx. 10.5 nmol protein/ml resin. Average recovery from seven successive purifications performed on the same column with EDTA elution was 90% with no regeneration and 93% with regeneration.

Number of times a column may be reused

Poured columns containing the affinity matrix may be used 7–10 times before a drop in yield of purified protein is observed. If a drop in yield is observed prior to the tenth column use and regeneration has not been performed, stripping the column may improve the yield.

Typical Results

Western blot (Fig. 1) and silver-stained gel (Fig. 2) demonstrating purification of Protein C-tagged β -galactosidase from a crude bacterial extract from both the N- and C-terminal positions. A 0.5-ml Anti-Protein C Affinity Matrix column was used to purify 3 mg of total protein



Fig 1: Lane 1: Crude bacterial extract; Lane 2: Column flow through; Lane 3: Purified protein

N-terminal C-terminal
1 2 3 4 5 6 7

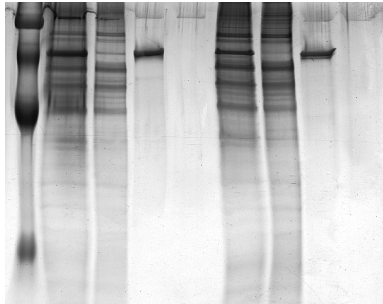


Fig 2: Lane 1: Molecular weight marker; Lane 2 and 5: Crude bacterial extract; Lane 3 and 6: Column flow through; Lane 4 and 7: Purified protein

2.3 Immunoprecipitation

Additional required equipment

- Microcentrifuge and 1.5 ml microcentrifuge tubes
- Pipettors
- Pipette tips (wide-bore and small-bore types)
- End-over-end rocker

Additional required reagents

Prepare the following reagents before beginning the immunoprecipitation procedure.

Buffer	Concentration
Cell lysis buffer	50 mM Tris, pH 7.5; 150 mM NaCl; 1 mM CaCl ₂ ; 1% Nonidet P-40, 0.5% deoxycholate; Complete Protease Inhibitor Cocktail Tablets, EDTA-free ⓘ Other buffers may be substituted. See notes regarding sample preparation (sec. 2.1)
Wash buffer	50 mM Tris, pH 7.5; 250 mM NaCl; 1 mM CaCl ₂ ; 0.1% Nonidet P-40; 0.05% deoxycholate
EDTA elution buffer	20 mM Tris, pH 7.5; 0.1 M NaCl, 5 mM EDTA

Immunoprecipitation procedure

- 1 Pipet 50–100 μ l of resuspended matrix into each microcentrifuge tube to be used for immunoprecipitation. Use wide-bore pipette tips.
- 2 Pellet matrix with a brief pulse in the microcentrifuge. Carefully remove supernatant using fine-bore pipette tip, then resuspend matrix in 0.5–1 ml cold Cell lysis buffer.
- 3 Repeat step 2, 1–2 more times.
 ⓘ Affinity matrix may be used directly without pre-rinsing; however, occasional Ig bands may be observed during western blotting.
- 4 Add 0.5–1.0 ml of cold crude protein extract to final matrix pellet.
- 5 Incubate samples at +2 to +8°C on rocker for 1 h to overnight.
 ⓘ Longer incubations increase binding to the affinity matrix.
- 6 Pellet matrix at full speed in microcentrifuge for 30 s. Carefully remove supernatant and rinse twice with 1 ml of cold Cell lysis buffer.
- 7 Perform final wash with 1 ml of Wash buffer, removing all residual buffer from pelleted matrix.

- 8 Use one of the following methods to elute tagged protein:
EDTA elution: Add 1–2 matrix-volumes (50–200 μ l) of EDTA elution buffer and rock at +2 to +8°C for 15–30 minutes. Pellet matrix, saving supernatant. Dilute supernatant in electrophoresis sample buffer or analyze as desired.
Direct elution into electrophoresis sample buffer: Add 1–2 matrix-volumes of sample buffer and boil for 5 min. Pellet matrix before gel electrophoresis.

2.4 Analysis of purification or immunoprecipitation samples by western blotting

Additional required reagents

Product	Cat. No.
PVDF Western Blotting Membranes	03 010 031 001
Anti-Protein C	11 814 508 001
Western Blocking Reagent	11 921 673 001
Lumi-Light Western Blotting Substrate	12 015 200 001
Lumi-Film Chemiluminescent Detection Film	11 666 657 001
Tween 20	11 332 465 001
Methanol	
Plastic wrap	
TBS	

Additional required buffers

Prepare the following reagents before beginning the western blotting procedure.

Buffer	Concentration
Transfer buffer	10% methanol, 24 mM Tris base, 194 mM glycine
Blocking buffer	Tris-buffered saline (10 mM Tris, pH 7.5; 150 mM NaCl), containing 1 mM CaCl_2 and 1 \times Western Blocking Reagent
TBS- Ca^{2+} solution	Tris-buffered saline (10 mM Tris, pH 7.5; 150 mM NaCl), containing 1 mM CaCl_2
TBST- Ca^{2+} solution	Tris-buffered saline (10 mM Tris, pH 7.5; 150 mM NaCl), containing 1 mM CaCl_2 and 0.05% Tween 20 Detergent

Western Blotting Procedure

- 1 Perform gel electrophoresis according to standard protocols (14). Wet PVDF membrane in 100% methanol and equilibrate the membrane in Transfer buffer. Perform western transfer to the PVDF membrane.
- 2 After transfer, block the membrane for 1 h at +15 to +25°C in Blocking buffer with gentle shaking.
Ⓢ 10 ml of Blocking buffer covers a 10 cm × 10 cm PVDF membrane
- 3 Combine 10 ml Blocking buffer with 10 ml TBS-Ca²⁺ solution.
- 4 Prepare working-strength anti-Protein C (final antibody concentration, 0.1–1 µg/ml) by diluting anti-Protein C stock solution with the solution prepared in step 3.
- 5 Incubate the blocked membrane with working-strength anti-Protein C for 1 h at +15 to +25°C with gentle shaking.
- 6 Wash the membrane three times, 5 min per wash, with 10 ml TBST-Ca²⁺ solution.
- 7 Prepare 10 ml working-strength anti-mouse IgG (H+L)-POD by diluting the secondary antibody 1 : 4,000 with the solution prepared in step 3. Add the secondary antibody and incubate the membrane for 30 min at +15 to +25°C with gentle shaking.
- 8 Wash the membrane three times, 5 min per wash, with 10 ml TBST-Ca²⁺ solution.
- 9 Prepare Lumi-Light Western Blot Substrate (POD) according to the directions in the package insert. Incubate membrane in this solution for 5 min.
- 10 Drain excess detection solution from membrane and wrap in plastic wrap. Expose the membrane to X-ray film for 60 s according to the method provided with the substrate.
Ⓢ Substrate development and X-ray film exposure conditions may vary for each experiment.

3. Troubleshooting

	Possible Cause	Recommendation
Little or no protein C tagged protein is eluted	<ul style="list-style-type: none"> • Tagged protein is degraded • Tagged protein not fully eluted • Tagged protein expression is absent • Tagged protein expression is very low 	<ul style="list-style-type: none"> • Include protease inhibitors and perform purification at +2 to +8°C. • If working at less than 37°C, increase temperature, time, and/or number of elutions. Try batch mixing of peptide solution with matrix. • Check for expression of protein in crude extract by western blot or biochemical assay. • Load larger volume of extract. Run column several times, pool, and concentrate final eluates.
Large quantities of tagged protein remain in the flow-through sample	<ul style="list-style-type: none"> • Column is overloaded • Column not regenerated after use 	<ul style="list-style-type: none"> • Decrease amount of loaded protein extract. • Regenerate column.
Column flow stops	<ul style="list-style-type: none"> • Column is overloaded • Starting extract contains insoluble materials • Air bubble in needle 	<ul style="list-style-type: none"> • Decrease amount of loaded protein extract. • Preclear starting extract by high-speed centrifugation or filtration. • Replace needle or place gentle pressure on column by briefly covering top of column with gloved hand.
Tagged protein appears degraded (a smear or multiple lower molecular weight bands on western blot)	<p>Protease activity during procedure</p>	<ul style="list-style-type: none"> • Increase protease inhibitors in protein extract sample. • Perform all steps at +2 to +8°C.
Detection of Ig heavy or light chain on western blot following immunoprecipitation	<p>Small quantities of heavy or light chain will accumulate over time in the matrix supernatant. This does not affect the performance (capacity) of the matrix before its expiration date.</p>	<p>Pre-rinse affinity matrix before using for immunoprecipitation.</p>

4. Additional Information on this Product

4.1 How this Product Works

Protein C is a Vitamin K-dependent plasma zymogen that is activated by proteolytic cleavage of the thrombin-thrombomodulin complex to form an anticoagulant enzyme (1,2). Anti-protein C mouse monoclonal antibody (clone HPC4) binds specifically to an epitope sequence spanning the thrombin cleavage site of protein C (3). The formation of the anti-protein C/protein C epitope tag complex is dependent on the presence of calcium, therefore, this unique antibody is especially well suited for use as a reagent in the purification of recombinant fusion proteins tagged with the protein C epitope (4-11).

In comparison to other Ca^{2+} -dependent systems for the expression and purification of recombinant fusion proteins (12), the protein C/anti-protein C epitope tagging system provides features that are beneficial for epitope tagging applications, particularly for immunoaffinity purification of epitope - tagged fusion proteins. These include:

- The protein C epitope does not contain the Ca^{2+} binding site. The HPC4 monoclonal antibody contains the Ca^{2+} binding site. Thus, insertion of the protein C tag into the fusion protein does not introduce a new calcium-(metal ion-) binding domain.
- The protein C tag can be integrated at either the N-terminus or the C-terminus without any change in antibody specificity. Thus, only one high-affinity ($K_D = 10^{-9}$ M) antibody (HPC4) is required for all immunochemical applications.
- Regardless of the location of the tag insertion, the protein C tag allows rapid immunoaffinity purification of tagged fusion proteins under non-denaturing elution conditions using economical calcium chelating reagents (*e.g.*, EDTA), or alternatively with a specific protein C-tag peptide.

4.2 References

- | | |
|--|--|
| Protein C | 1 Esmon, C.T. (1987) The regulation of natural anticoagulant pathways. <i>Science</i> 235 , 1346–1352. |
| | 2 Kisiel, W., and Davie, E.W. (1981) <i>Protein C. Methods Enzymol.</i> 80 , 320–332. |
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| Unique Properties of Anti-Protein C for Immunoaffinity Purification | 4 Rezaie A.R., <i>et al.</i> (1992) Expression and purification of a soluble tissue factor fusion protein with an epitope for an unusual calcium-dependent antibody. <i>Protein Expression and Purification</i> 3 , 453–460. |
| | 5 Fiore M.M., <i>et al.</i> (1992) An unusual antibody that blocks tissue factor/factor VIIa function by inhibiting cleavage only of macromolecular substrates. <i>Blood</i> 80 , 3127–3134. |
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| | 9 Rezaie A.R., and Esmon, C.T. (1994) Asp-70/E Lys mutant of factor X lacks high affinity Ca ²⁺ binding site yet retains function. <i>J. Biol. Chem.</i> 269 , 21495–21499. |
| | 10 Rezaie A.R., and Esmon, C.T. (1994) Proline at the P2 position in protein C is important for calcium-mediated regulation of protein C activation and secretion. <i>Blood</i> 83 , 2526–2531. |
| | 11 Zheng, Z. <i>et al.</i> (1995) Monoclonal antibodies to CD44 and their influence on hyaluronan recognition. <i>J. Cell Biol.</i> 130 , 485–495. |
| Limitations of other Ca²⁺ Dependent Purification | 12 Prickett, K.S. <i>et al.</i> (1989) A calcium-dependent antibody for identification and purification of recombinant proteins. <i>BioTechniques</i> 7 , 580–589. |
| Sample Preparation | 13 Harlow, E. and Lane, D. (1988) <i>Antibodies, A Laboratory Manual</i> , Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y. |
| Electrophoresis Methods | 14 Dunn, M.J. (1989) <i>Advances in electrophoresis</i> , (Radola, B.J., Dunn, M.J. & Chrambach, A., eds.) VCH Verlagsgesellschaft, Weinheim, Vol. 1. |

4.3 Quality Control

Each lot of Anti-Protein C Affinity Matrix is tested for its ability to purify a protein C-tagged protein expressed in transformed bacteria from a crude bacterial extract. The antibody affinity column is used in combination with gel electrophoresis, followed by western blot and/or silver-stain analysis.

5. Supplementary Information

5.1 Conventions

Text Conventions To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed
Numbered instructions labeled 1, 2, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
ⓘ	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

- Editorial changes.

5.3 Ordering Information

Product	Pack Size.	Cat. Nos.
Anti-HA, High Affinity (3F10) lyophilized, unconjugated	50 µg	11 867 423 001
lyophilized, unconjugated peroxidase	500 µg 25 U (25 µg)	11 867 431 001 12 013 819 001
Anti-HA (12CA5) lyophilized, unconjugated	200 µg	11 583 816 001
in solution, unconjugated	5 mg (1 ml)	11 666 606 001
biotin	100 µg (500 µl)	11 666 851 001
fluorescein	100 µg (500 µl)	11 666 878 001
rhodamine	100 µg (500 µl)	11 666 959 001
peroxidase	50 µg (500 µl)	11 667 475 001

Product	Pack Size.	Cat. Nos.
HA Peptide	5 mg	11 666 975 001
Streptavidin-POD	500 U (1 ml)	11 089 153 001
Anti-Biotin-POD, Fab fragments	150 U	11 426 311 001
DOSPER Liposomal Transfection Reagent	2 ml (5 x 0.4 ml) 0.4 ml	11 781 995 001 11 811 169 001
DOTAP Liposomal Transfection Reagent	2 ml (5 x 0.4 ml) 0.4 ml	11 202 375 001 11 811 177 001
FuGENE [®] 6 Transfection Reagent	0.4 ml 1 ml 5 × 1 ml Mega-pack 5 × 1 ml, packed together 10 ml	11 815 091 001 11 814 443 001 11 815 075 001 11 988 387 001 05 061 377 001
FuGENE [®] HD Transfection Reagent	0.4 ml 1 ml Mega-pack 5 × 1 ml 10 ml Trial-pack	04 709 691 001 04 709 705 001 04 709 713 001 04 709 713 001 04 883 560 001
Tween [®] 20	5 × 10 ml	11 332 465 001
PVDF Western Blotting Membranes	1 roll (30 cm × 3 m) 10 sheets (15 × 15 cm)	03 010 040 001 03 010 031 001
Lumi-Light Western Blotting Substrate	400 ml (4000 cm ² membrane)	12 015 200 001
Lumi-Light ^{PLUS} Western Blotting Substrate	100 ml (1000 cm ² membrane)	12 015 196 001
Lumi-Light ^{PLUS} Western Blotting Kit (Mouse/Rabbit)	1000 cm ² membrane	12 015 218 001
Lumi-Film Chemiluminescent Detection Film	100 films (8 × 10 inches 20.3 × 25.4 cm)	11 666 657 001

Product	Pack Size.	Cat. Nos.
cOmplete Protease Inhibitor Cocktail Tablets	20 tablets (each sufficient for 50 ml extract)	11 697 498 001
	3 × 20 tablets	11 836 145 001
cOmplete, Mini Protease Inhibitor Cocktail Tablets	25 tablets (each sufficient for 10 ml extract)	11 836 153 001
Anti-Protein-C lyophilized, unconjugated in solution, unconjugated peroxidase conjugate	200 µg	11 814 508 001
	5 mg (1 ml)	11 814 516 001
	50 µg	11 814 974 001
Anti-c-myc lyophilized, unconjugated in solution, unconjugated peroxidase	200 µg	11 667 149 001
	5 mg	11 667 203 001
	500 µg (500 µl)	11 814 150 001
c-myc peptide	5 mg	11 667 246 001
Anti-His ₆ -Peroxidase	100 µg	11 922 416 001
Anti-GFP	200 µg	11 814 460 001
rGFP	50 µg	11 814 524 001

5.4 Trademarks

COMPLETE is a trademark of Roche.

All third party product names and trademarks are the property of their respective owners.

5.5 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.6 Disclaimer of Licence

For patent license limitations for individual products please refer to:
[List of biochemical reagent products](#)

Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.

Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

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