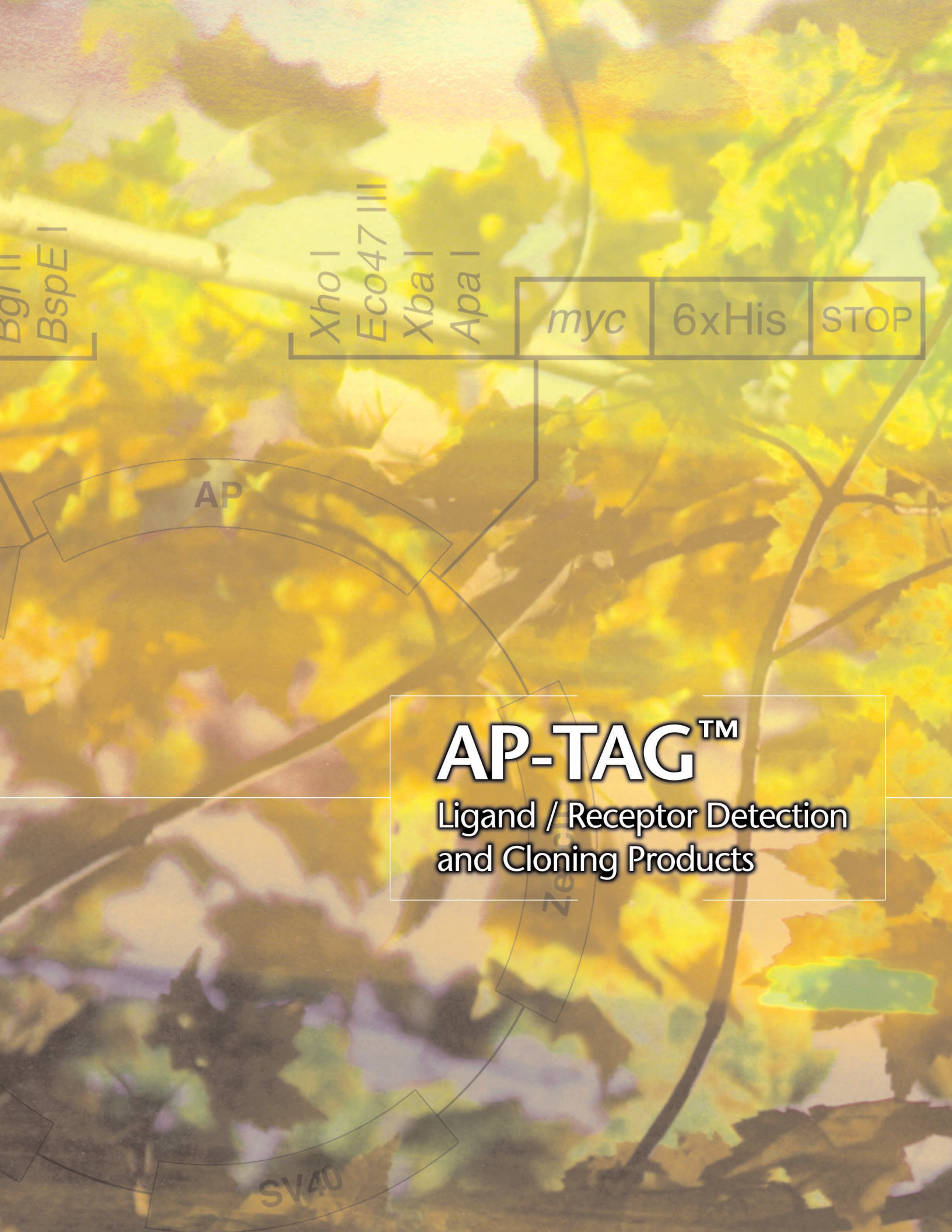


AP-TAG™

Ligand / Receptor Detection and Cloning Products

AP-TAG® Introduction

AP-TAG® technology (US patents 5,554,499 and 5,801,000; ref. 1), invented by Drs. J. Flanagan and P. Leder at Harvard Medical School, has revolutionized the way cell surface receptors and ligands are detected and cloned. GenHunter is proud to be the exclusive licensee of this powerful method. Purchase of an AP-TAG® Kit or any pAPtag vector comes with a limited, single-user and non-transferable sublicense for use in research applications only. No part of the kit or pAPtag plasmid vectors shall be disseminated, propagated or distributed outside the user's own laboratory without written permission from GenHunter. A separate license is required for drug screening or other commercial applications. Contact GenHunter for details.

The essence of this invention is to allow a cDNA sequence encoding any secreted polypeptide ligand or extracellular domain of a receptor to be in-frame fused to human placental secreted alkaline phosphatase (AP) in pAPtag cloning vectors. The resulting AP fusion protein, designated as an AP-body™, when expressed in 293T cells, can be secreted at high levels into the culture medium and thus easily detected by either the AP activity assay or Western blot analysis using antibody against AP. The ligand-AP or soluble receptor-AP fusion proteins thus can serve as affinity agents much like antibodies, which allow the most convenient, safe, and sensitive detection and cloning of their corresponding cell surface receptors or ligands. Unlike the conventional radioactive ^{125}I labeling method, AP-TAG® is safe and does not require ligand/soluble receptor purification.

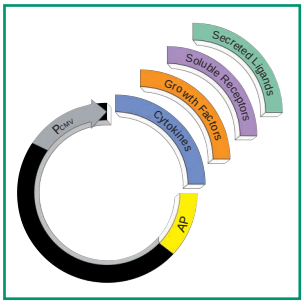
Since its invention, many important cell surface receptors and ligands have been cloned by AP-TAG™ technology including receptors for **Leptin**, **Semaphorin III**, **Nogo-66**, **IL-24**, **Jelly Belly**, and ligands for **Kit**, **Mek4** and **Sek receptor tyrosine kinases** (see references on page 46). A more extensive list of publications using AP-TAG® technology can be found on page 59 or on our website.

GenHunter was extremely pleased to be able to add this innovative method into our product line as a powerful tool for applications downstream of differential display (DD). If you are working with a secreted protein or cell surface molecule cloned by DD or other methods, AP-TAG® technology may allow you to functionally characterize these genes further.

Comparison of AP-TAG® technology and the conventional radioactive ^{125}I ligand-labeling method:

	AP-TAG®	^{125}I labeling
Ligand purification	Not required	Required
Labeling Reaction	Not required	Required
Hazardous	No	Yes
Detection	Colorimetric	Scintillation counting
Sensitivity	High	High
Cell Staining	Yes	No
Expression Cloning	Yes	Yes
Ligand-Receptor Binding Kinetics/Affinity	Yes	Yes

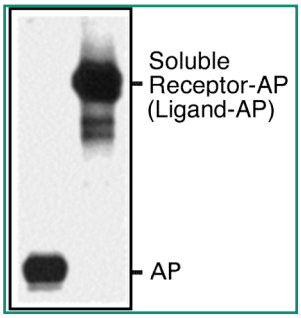
Schematic Illustration of AP-TAG® technology and its major applications



AP fusion construct

Create an in-frame fusion of your cDNA encoding a secreted ligand or soluble receptor with either the N- or C-terminus of secreted alkaline phosphatase (AP) in pAPtag expression vectors.

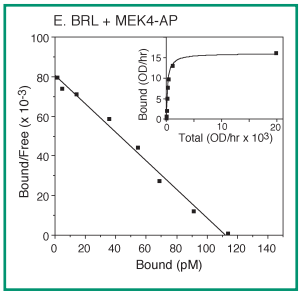
GenHunter Products: AP-TAG® Kit A
AP-TAG® Kit B



AP fusion protein expression

After transfecting the above AP fusion plasmid construct into 293T or NIH 3T3 cells, the expression of the secreted AP fusion protein (AP-body™) can be measured by either colorimetric AP activity assay or immunoblotting (or IP) with antibody to AP.

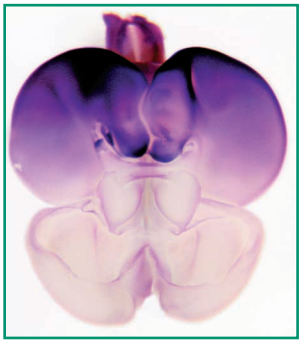
GenHunter Products: 293T Cells
AP Antibody (Polyclonal and Monoclonal)
AP Assay Reagent A
Monoclonal AP Antibody Sepharose Beads



Receptor/ligand binding assay

The culture medium containing the secreted AP fusion protein can be used directly to measure the presence or absence of a cell surface receptor (or ligand) of interest by assaying the AP activity bound to the cells. The secreted AP alone is used as a negative control.

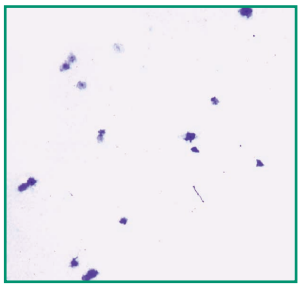
GenHunter Products: AP Assay Reagent A
293T/pAPtag-4 stable cell line
AP control AP-body™



in situ staining of receptor/ligand

The secreted AP fusion protein can be used much like an antibody to detect the tissue distribution of a cell surface receptor/ligand of interest. An expression cDNA library thus can be made with mRNA isolated from tissues that express the highest level of the receptor/ligand for subsequent expression cloning.

GenHunter Products: AP Assay Reagent S



Expression cloning of receptor/ligand

The secreted AP fusion protein can be used as a probe to clone a cell surface receptor or ligand of interest by traditional expression cloning strategy (panning).

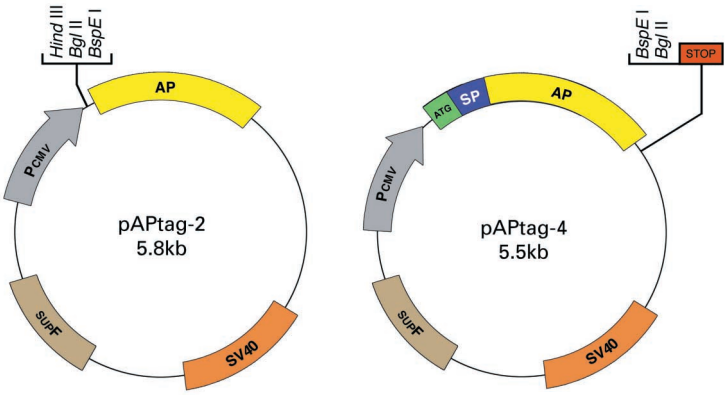
GenHunter Products: Expression Cloning Kit
AP Assay Reagent S
Kit-AP AP-body™
Kit Ligand Positive Control Vector
pMT21 Expression Vector

AP-TAG® Kit A

For Academic/Non-Profit: Cat. No.: Q201 Price: $980
For Industry: Cat. No.: Q201P Price: $3400

For non-radioactive detection of receptor/ligand interaction

This is the second generation of AP-TAG® technology. A secreted ligand or soluble receptor can be fused with secreted alkaline phosphatase (AP) at either its N- or C-terminus to produce an "AP-body™". The resulting AP fusion protein can be expressed as a secreted protein and used directly as highly sensitive affinity agents much like an antibody.



Features	pAPtag-2	pAPtag-4
Size (kb)	5.8	5.5
AP fusion Type	Ligand-AP (Receptor-AP)	AP-Ligand (AP-Receptor)
Cloning Sites	*Hind* III, *Bgl* II, *BspE* I	*Bgl* II, *BspE* I
Promoter	CMV	CMV
SV40 Ori.	Yes	Yes
E. coli Host	GH2/P3	GH2/P3
Vector selection	Tet/Amp	Tet/Amp
Secretion Signal	From insert	From AP
AP negative Control (secretes AP alone)	No	Yes

AP-TAG® Kit A

1. pAPtag-2 (10 µg)	40 µL
2. pAPtag-4 (10 µg)	40 µL
3. L-AP Primer (2 µM)	100 µL
4. R-AP Primer (2 µM)	100 µL
5. Colony lysis buffer	2 X 1 mL

NOTE: pAPtag-2 and pAPtag-4 plasmid vectors can only be transformed or propagated in E. coli host cells with P3 episome such as **GH2/P3 Supercompetent cells (Cat. No. T601)**.

The L(left)- and R(right)-AP primers flanking the cloning sites of pAPtag-2 are used in PCR to check for the presence and size of DNA insert cloned into the vector and for sequence verification of the Ligand-AP or soluble receptor-AP fusion constructs. The L-AP4 and R-AP4 primers (not included in kit) flank the cloning sites of pAPtag-4 and can be purchased separately (see below).

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.

References:

1. Flanagan, J. G. and Leder, P. (1990). The kit ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.
2. Cheng, H.J., and Flanagan, J.G. (1994). Identification and cloning of ELF-1, a developmentally expressed ligand for Mek4 and Sek receptor tyrosine kinases. ***Cell*** 79, 157-168.
3. Tartaglia, L.A. et al. (1995). Identification and expression cloning of a leptin receptor, OB-R. ***Cell*** 83, 1263-1271.
4. He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. ***Cell*** 90, 739-751.
5. Flanagan, J.G., et al. (2000). Alkaline phosphatase fusions of ligands or receptors as *in situ* probes for staining of cells, tissues and embryos. ***Methods in Enzymology*** 327, 17-35.
6. Flanagan, J.G., and Cheng, H.-J. (2000). Alkaline phosphatase fusion proteins for molecular characterization and cloning of ligands and receptors. ***Methods in Enzymology*** 327, 198-210.
7. US patents 5,554,499 and 5,801,000.

See page 59 for an extensive list of AP-TAG® References.

Individual components for the AP-TAG® Kit A sold separately:

Description	Volume	Cat. No. Academic	Cat. No. Industry	Price Academic	Price Industry
pAPtag-2 (10 µg)	40 µL	QV2	QV2P	$960	$3390
pAPtag-4 (10 µg)	40 µL	QV4	QV4P	$960	$3390
L-AP Primer (2 µM)	100 µL	Q210	Q210	$55	$55
R-AP Primer (2 µM)	100 µL	Q211	Q211	$55	$55
L-AP4 Primer (2 µM)	100 µL	Q213	Q213	$55	$55
R-AP4 Primer (2 µM)	100 µL	Q214	Q214	$55	$55
Colony Lysis Buffer	5 mL	L102	L102	$59	$59

AP-TAG® Kit B

For Academic/Non-Profit: Cat. No.: Q202 Price: $980
For Industry: Cat. No.: Q202P Price: $3400

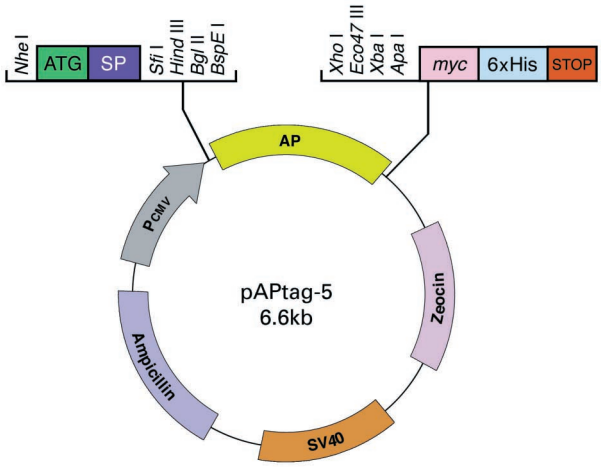
For non-radioactive detection of receptor/ligand interaction

This single vector system is the third generation AP-TAG® technology. A secreted ligand or soluble receptor can be fused with secreted alkaline phosphatase (AP) at either its N- or C-terminus to produce an "AP-body™". The resulting AP fusion protein can be expressed as a secreted protein and used directly as highly sensitive affinity agents much like an antibody. The epitope tags (6xHis and *myc*) allow easy purification, detection and interaction assays (IP) of the AP-fusion proteins. Other improved features are listed below.

Features	pAPtag-5
Size (kb)	6.6
AP fusion Type	Ligand-AP or AP-Ligand (Receptor-AP or AP-Receptor)
Cloning Sites	*Sfi* I, *Hind* III, *Bgl* II, *BspE* I or *Xho* I, *Eco47* III, *Xba* I *Apa* I
Promoter	CMV
SV40 Ori.	Yes
E. coli Host	GH (no need for P3)
Vector Selection	Ampicillin
Secretion Signal	From vector or insert
AP negative Control (secretes AP alone)	Yes
Affinity Tags	6xHis and *myc*
Transfection marker	Zeocin (Invitrogen)

AP-TAG® Kit B

1. pAPtag-5 (20 µg)	40 µL
2. L-AP5 Primer (2 µM)	100 µL
3. R-AP Primer (2 µM)	100 µL
4. Colony lysis buffer	2 X 1 mL



NOTE: pAPtag-5 plasmid vector can be transformed or propagated in **GH Competent cells (Cat. No. L301)**.

The L(left)-AP5 and R(right)-AP primers flank the N-terminal cloning site of pAPtag-5 and are used to check for the presence and size of DNA insert cloned into the vector and for sequence verification of the Ligand-AP or soluble receptor-AP fusion constructs (N-terminal of AP only). For C-terminal cloning, the L-AP5C and R-AP5C primers (not included in the kit) can be used (see below).

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.

References:

1. Flanagan, J.G., et al. (2000). Alkaline phosphatase fusions of ligands or receptors as *in situ* probes for staining of cells, tissues and embryos. ***Methods in Enzymology*** 327, 17-35.
2. Flanagan, J.G., and Cheng, H.-J. (2000). Alkaline phosphatase fusion proteins for molecular characterization and cloning of ligands and receptors. ***Methods in Enzymology*** 327, 198-210.
3. US patents 5,554,499 and 5,801,000.

See page 59 for an extensive list of AP-TAG® References.

Individual components for the AP-TAG® Kit B sold separately:

Description	Volume	Cat. No. Academic	Cat. No. Industry	Price Academic	Price Industry
pAPtag-5 (20 µg)	40 µL	QV5	QV5P	$960	$3390
L-AP5 Primer (2 µM)	100 µL	Q212	Q212	$55	$55
R-AP Primer (2 µM)	100 µL	Q211	Q211	$55	$55
L-AP5C Primer (2 µM)	100 µL	Q215	Q215	$55	$55
R-AP5C Primer (2 µM)	100 µL	Q216	Q216	$55	$55
Colony Lysis Buffer	5 mL	L102	L102	$59	$59

GH2/P3 Supercompetent Cells

Cat. No.: T601 **Price: $210**

For transformation of pAPtag-2 and pAPtag-4 vectors

The pAPtag-2 and pAPtag-4 AP-fusion cloning vectors contain the supF gene which confers both ampicillin and tetracycline resistance when transformed into the GH2/P3 Supercompetent Cells. pAPtag-2 and pAPtag-4 vectors will not confer antibiotic resistance in an *E. coli* host which does not contain the P3 episome. A tube of 1000X AT antibiotics mix is included for your convenience to prepare ampicillin (25 µg/mL) and tetracycline (10 µg/mL) plates for 1 L of LB-agar.

Detailed protocol included.

GH2/P3 Supercompetent Cells

1. GH2/P3 Supercompetent Cells	5 x 0.4 mL
2. 1000X AT Antibiotics Mix (Amp and Tet)	1 mL

AT Antibiotics Mix (1000X)

Cat. No.: Q601 **Size: 1 mL** **Price: $43**

For selection of pAPtag-2 and pAPtag-4 plasmids

Each tube of AT antibiotics mix is conveniently packaged to prepare ampicillin (25 µg/mL) and tetracycline (10 µg/mL) plates for 1 L of LB-agar.

GH Competent Cells

Cat. No.: L301 **Size: 6 x 0.5 mL** **Price: $210**

For transformation of pAPtag-5 vector

The pAPtag-5 AP fusion cloning vector contains the ampicillin resistance gene. It can be easily and efficiently transformed and propagated in GH Competent cells.

293T/pAPtag-4 Stable Cell Line

Cat. No.: Q402 **Size: 5 x 10^6 Cells / Vial** **Price: $309**

For production of high levels of AP alone

The 293T/pAPtag-4 stable cell line is used to produce high levels of secreted human placental alkaline phosphatase (AP) which can be used as a negative control for a ligand-AP or soluble receptor-AP fusion protein in cell surface binding assays or cell staining. High level production of secreted AP can be achieved with sub-confluent to confluent culture a few days after medium change. The secretion of AP can be monitored easily with the culture medium by AP activity assay using GenHunter AP Assay Reagent A (Cat. No. Q501).

Detailed protocol included.

293T Cells

Cat. No.: Q401 **Size: 5 x 10^6 Cells / Vial** **Price: $236**

For transfection with pAPtag vectors

293T is a human embryonic kidney (HEK) cell line commonly used for transfection assays. Due to the expression of the large T antigen in the cells, plasmids with SV40 origin of replication (such as pAPtag-2, pAPtag-4, and pAPtag-5) can be transiently transfected and give extremely high levels of expression of AP fusion proteins (e.g. ligand-AP fusion proteins). Thus, we strongly recommend using this cell line for your production of AP fusion proteins with pAPtag vectors. The fusion proteins can be easily monitored 2-3 days after transfection by alkaline phosphatase assay (See our AP Assay Reagent A, Cat. No. Q501) or by Western blot using AP Antibody. But for long term production of AP fusion proteins, we recommend that a stable cell line be cloned by co-transfecting with a puromycin or hygromycin-resistant plasmid (293T is G418 resistant). See below for information on the co-transfection vectors we offer. ***Detailed protocol included.***

293T-S Cells (for Serum Free)

Cat. No.: Q401-S **Size: 5 x 10^6 Cells / Vial** **Price: $318**

For transfection with pAPtag vectors for serum free production

The 293T-S cell line is a clone of the same HEK 293T cell line (above), but it has already been adapted for use in serum-free (SFM) production. They can be used with the pAPtag-2, pAPtag-4, and pAPtag-5 vectors as well. Just like the standard 293T cells, the fusion proteins can be easily monitored after transfection by alkaline phosphatase assay or by Western blot using AP Antibody. But for long term production of AP fusion proteins, we recommend that a stable cell line be cloned using a co-transfection vectors (see below). ***Detailed protocol included.***

GH-CHO (DHFR-) Cells

Cat. No.: Q420 **Size: 5 x 10^6 Cells / Vial** **Price: $247**

For transfection with DHFR vectors

GenHunter's Chinese Hamster Ovary (CHO) cells are dihydrofolate reductase deficient (DHFR-) and allow for high-level expression of recombinant proteins in suspension or attached cultures. The cell line is developed for rapid growth and ease for adaptation in serum free culture. ***Detailed protocol included.***

DHFR Vectors

For cloning, expression, & gene amplification of recombinant proteins

The pAPtag-2-DHFR and pDHFR vectors allow for high-level expression of recombinant proteins in suspension or attached cultures using the GH-CHO (DHFR-) cells (Cat. No. Q420 - above).

Vector	Cat. No.	Price
pAPtag-2-DHFR vector	QVD2	$2600
pDHFR vector (DHFR expression cassette)	Q430	$1059

Co-transfection Vectors

For use as a selectable marker for transfection of cultured mammalian cells.

The pSV2-Hygro or pBabe-Hygro vectors confer hygromycin resistance and the pBabe-Puro vector confers puromycin resistance when co-transfected into cells.

Vector	Selectable Marker	Cat. No.	Volume	Price
pSV2-Hygro co-transfection vector	Hygromycin	Q455	stab	$163
pBabe-Hygro co-transfection vector	Hygromycin	Q455-B	stab	$163
pBabe-Puro co-transfection vector	Puromycin	Q456	10 µg	$163

AP Western Blot Kit

Cat. No.: Q310 **Price: $414**

For immunoblotting of AP fusion proteins

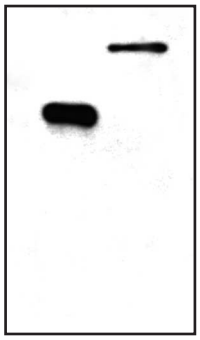
This kit contains the AP Antibody (rabbit polyclonal) which is specific to human placental secreted AP as well as two controls for AP Western blots. The antibody works optimally when used for Western blot analysis of secreted AP fusion proteins from culture media. It should be noted that although this antibody works extremely well for Western blot detection of AP fusion proteins, AP itself (with a MW of 67 KD) may not be detected directly from the culture media due to the amount of albumin which runs at a similar MW. Therefore, the purified AP from human placenta and a known soluble receptor AP fusion protein are provided as positive controls for the antibody. In addition, this antibody only recognizes the denatured form of AP. The Monoclonal AP Antibody (Cat. # Q320) can be used for applications where recognition of the native form is required.

Detection Limit: 20 mU of AP

AP Western Blot Kit

1. AP Antibody (human placenta) - Polyclonal From rabbit	100 µL
2. Purified AP (Western blot control) human placenta, 1 unit/mL	100 µL
3. Kit-AP fusion protein control media 1unit/mL	200 µL

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.



— **Kit-AP (150 KD)**

— **AP (67 KD)**

Western blot analysis of AP fusion proteins (antibody dilution 1:2000)

Individual components for the AP Western Blot Kit sold separately:

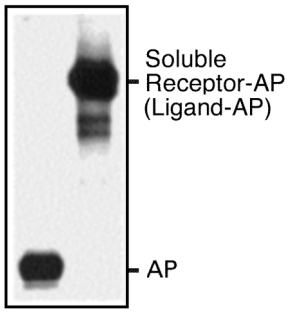
Description	Cat. No.	Volume	Price
AP Antibody (human placenta) - Polyclonal	Q301	100 µL	$295
Purified AP (Western blot control)	Q302	100 µL	$73
Kit-AP fusion protein media (positive control)	Q303	200 µL	$73

AP Antibody (human placenta) - Monoclonal

For ELISA Assay of Alkaline Phosphatase or IP (Immunoprecipitation)

This Monoclonal AP Antibody (human placental) is purified IgG 2a with a concentration of approximately 2.3 mg/mL. It is purified by DEAE chromatography and is in 15mM potassium phosphate buffer, 150mM sodium chloride, 0.1% sodium azide, pH 7.2. This antibody does not recognize denatured AP and therefore cannot be used for Western Blot directly.

	Cat. No.	Volume	Price
AP Antibody (human placenta) - Monoclonal	Q320	100 µg	$323
AP Antibody (human placenta) - Monoclonal	Q320-5	500 µg	$982
AP Antibody (human placenta) - Monoclonal	Q321	1 mg	$1596



IP with Monoclonal AP Antibody

Monoclonal AP Antibody-Sepharose Beads

For one-step purification of AP-fusion proteins and proteins interacting with AP-fusion proteins

Applications:

1) Concentrating AP fusion protein from the conditioned media
2) Purification of AP fusion proteins
3) Affinity column purification of protein(s) or molecules interacting with the ligand/receptor-AP fusion protein
4) IP Western analysis of the AP fusion proteins (see Polyclonal AP Antibody)

Technical parameters:

Coupling condition: 1 mg of pure IgG/mL of beads

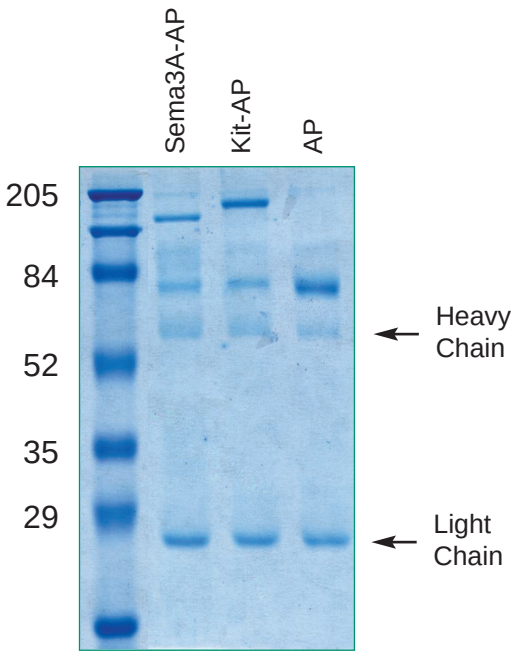
AP binding capacity: up to 200 Units/mL of beads

Specificity of the antibody: Native human placental AP

Interference of AP activity: The antibody binding does not interfere with the AP activity

Product Form:

The Monoclonal AP antibody-sepharose beads are supplied in a 50% suspension in 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.4.



Coomassie blue stain of different secreted AP-fusion proteins purified with our Monoclonal AP Antibody-Sepharose Beads.

	Cat. No.	Total Volume	Price
AP Antibody-Sepharose Beads	Q330	100 µL	$323
AP Antibody-Sepharose Beads	Q331	500 µL	$1249
AP Antibody-Sepharose Beads	Q332	1 mL	$1828

Antigen Elution Solution

For eluting AP fusion protein from the Monoclonal AP Antibody-Sepharose Beads

The Antigen Elution Solution is specially formulated for eluting AP or AP fusion proteins from the monoclonal AP Antibody-sepharose beads. This solution breaks the extremely tight interaction between the antibody-antigen complex, allowing up to 80% recovery of the bound antigen.

It is available in both Acidic and Basic versions, depending on the pH sensitivity of your protein.

	Cat. No.	Volume	Price
Antigen Elution Solution (Acidic)	Q340A	10 mL	$45
Antigen Elution Solution (Acidic)	Q341A	50 mL	$111
Antigen Elution Solution (Basic)	Q340B	10 mL	$45
Antigen Elution Solution (Basic)	Q341B	50 mL	$111

Expression Cloning Kit

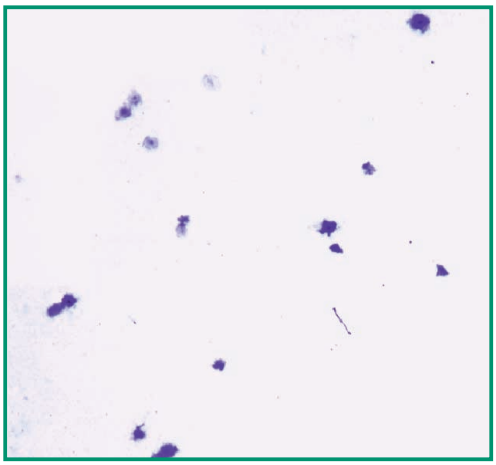
CAT. NO.: Q450 **PRICE: $433**

For expression cloning of cell surface receptor/ligand using AP fusion proteins (AP-bodies™)

This kit consists of a clonally purified cos-1 host cell line ideal for expression cloning by panning and a positive control receptor/ligand-AP pair.

An expression cDNA library potentially containing the receptor/ligand gene of interest can be transiently transfected into these cells. Positive cDNA pools can be identified by staining the transfected cells with your AP fusion protein. Cells over-expressing the corresponding cell surface receptor/ligand will be stained blue with GenHunter AP Activity Assay Reagent S (see figure below).

The Kit ligand positive control vector [containing a 1 kb cDNA encoding the transmembrane form of Kit ligand (stem cell factor), Ref. # 1 below] can be transfected into the cos-1 host cell line. Cells overexpressing cell surface Kit ligand will be stained blue by soluble receptor Kit-AP fusion protein using GenHunter AP Assay Reagent S.



Expression cloning of cell surface receptor/ligand by panning

Expression Cloning Kit

1. cos-1 Host Cell Line	1 x 10^6 cells / vial
2. Kit-AP fusion protein (media) 1 unit/mL	10 mL
3. Kit ligand (stem cell factor) positive control vector	10 µg

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.

References:

1. Flanagan, J.G. *et al*. (1991). Transmembrane Form of the *kit* Ligand Growth Factor is Determined by Alternative Splicing and is missing in the SI^d Mutant. ***Cell*** 64, 1025-1035.
2. Cheng, H.J., and Flanagan, J.G. (1994). Identification and cloning of ELF-1, a developmentally expressed ligand for Mek4 and Sek receptor tyrosine kinases. ***Cell*** 79, 157-168.
3. Tartaglia, L.A. *et al*. (1995). Identification and expression cloning of a leptin receptor, OB-R. ***Cell*** 83, 1263-1271.
4. He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. ***Cell*** 90, 739-751.
5. Wang, M., Tan, Z., Zhang, R., Kotenko, S.V. and Liang, P.: Interleukin-24 (Mob-5/Mda-7) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. ***J. Biol. Chem.*** 277, 7341-7347.

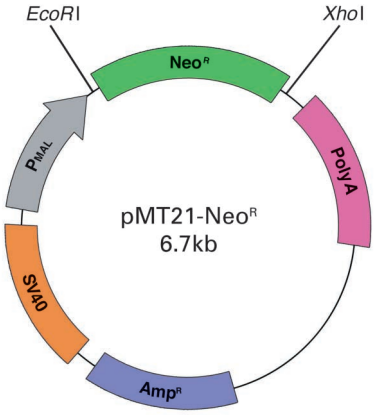
Individual components for the Expression Cloning Kit sold separately:

DESCRIPTION	CAT. NO.	VOLUME	PRICE
cos-1 Host Cell Line	Q451	1 x 10^6 cells/vial	$203
Kit-AP fusion protein (media), 1 unit/mL	Q452	10 mL	$107
Kit ligand (stem cell factor) positive control vector	Q453	10 µg	$201

pMT21-Neo Mammalian Expression Cloning Vector

CAT. NO.: Q454 **PRICE: $453**

For construction of expression cDNA libraries.



This cloning vector has been used extensively to construct mammalian expression cDNA libraries (see below references). The vector contains an SV40 origin of replication and the major adeno late promoter (P_{MAL}) in front of Neo resistance cDNA insert flanked by an *EcoR* I and *Xho* I site. Using the Stratagene cDNA Synthesis Kits generally results in cDNA ends with *EcoR* I and *Xho* I sites, which can be directionally cloned into the pMT21-Neo^R vector.

Amount:	20 µg
Cloning Sites:	*EcoR* I, *Xho* I (the excised Neo^R insert is 2kb)
Promoter:	Major Adeno Late Promoter (P_{MAL})
Antibiotic Resistance:	Ampicillin

References:

1. He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. ***Cell*** 90, 739-751.

2. Kolodkin, A.L. *et al.* (1997). Neuropilin is a Semaphorin III receptor. ***Cell*** 90, 753-762.

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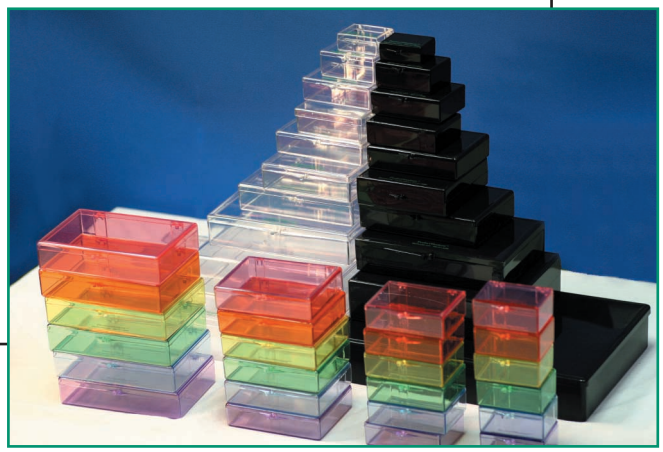


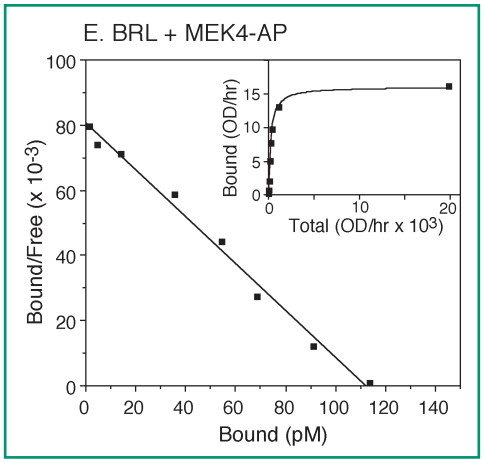
AP Assay Reagent A (For 200 Reactions)

Cat. No.: Q501 **Size: 10 mL** **Price: $128**

For AP Activity Assay

The AP assay reagent A is formulated specifically for measuring the enzymatic activity of the alkaline phosphatase (AP). The secretion of human placenta AP or a secreted ligand(receptor)-AP fusion protein can be easily monitored by this assay using the culture media in which the transfected cells are grown. The dephosphorylation of the p-Nitrophenyl phosphate by AP leads to the generation of yellow color which serves as both qualitative (by eye) and quantitative (Absorbance 405 nm) measurement of AP activity.

Detailed protocol included.



Receptor/Ligand Binding Assay using AP Assay Reagent A

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.

AP Assay Reagent S

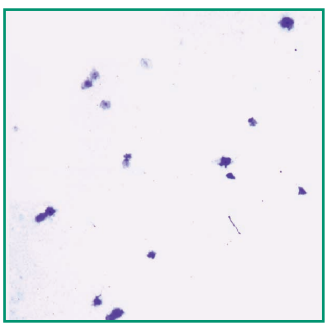
Cat. No.: Q502 **Size: 10 mL** **Price: $51**
Cat. No.: Q502L **Size: 100 mL** **Price: $359**

For Cell Staining

The AP Assay Reagent S is formulated specifically for cell staining or affinity blotting analysis of enzymatic activity of the alkaline phosphatase (AP). The AP substrate BCIP upon dephosphorylation forms an insoluble blue precipitate, thus it can be used for tissue or cell staining for the presence of receptors to which the ligand-AP fusion proteins bind. This assay kit can also be used during expression cloning of a receptor (Cheng and Flanagan, 1994, Cell 79:157-168).

Detailed protocol included.

***in situ* staining of receptor/ligand** (embryonic chick brain)



Expression cloning of receptor/ligand by panning

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.
2. Wang, M., Tan, Z., Zhang, R., Kotenko, S.V. and Liang, P. (2002). Interleukin-24 (Mob-5/Mda-7) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. ***J. Biol. Chem.*** 277, 7341-7347.
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HBHA Wash Buffer

For Receptor Binding Assay

HBHA wash buffer consists of Hank's balanced salt solution with 0.5 mg/mL BSA and 20 mM HEPES, pH 7.0. This buffer has been used extensively for ligand-receptor binding assays.

Detailed protocol included.

	Cat. No.	Volume	Price
HBHA Wash Buffer	Q503S	100 mL	$56
HBHA Wash Buffer	Q503L	500 mL	$203

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.

Cell Lysis Buffer

Cat. No.: Q504 **Size: 100 mL** **Price: $56**

For Cell Lysis in Receptor Binding Assay

The cell lysis buffer is used in ligand-receptor binding assay. This buffer allows rapid lysis of the cells and removal of cell nuclei before bound AP activity is measured.

Detailed protocol included.

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.

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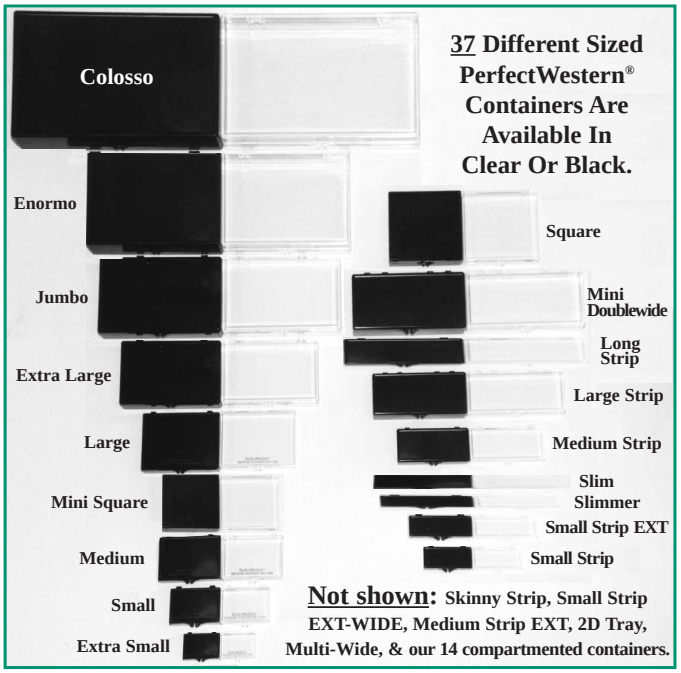
See next page for more information including dimensions, catalog numbers, and prices. **We highly recommend downloading or requesting the complete 2015 PerfectWestern brochure including a real-size layout of all 37 sizes at *www.PerfectWestern.com*.**



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Gel Manufacturer	Our Container	Cat. No.
Biometra (formerly from Invitrogen)		
Mini-V 8•10	Medium	B101
V15•17 Vert. Gel. Elect.	Jumbo/Enormo	B138/B111
V16 or V16-2	Jumbo/Enormo	B138/B111
Maxigel	Enormo/Colosso/2D Tray	B111/B140/B142
Bio-Rad Systems		
Mini-PROTEAN 3	Medium	B101
Criterion Precast	Extra Large	B109
PROTEAN II xi, 16cm	Jumbo/Enormo	B138/B111
PROTEAN II xi, 20cm	Enormo	B111
PROTEAN II XL, 20cm	Enormo/Colosso/2D Tray	B111/B140/B142
PROTEAN Plus	Colosso/2D Tray	B140/B142
GE Healthcare / Hoefer (formerly Amersham)		
Mighty Small SE250	Medium/Mini Square	B101/B144
Mighty Small SE260	Mini Square/Large	B144/B107
miniVE Vert. Gel Elect.(SE300)	Large/Square	B107/B119
SE600/SE400 (18 x 8)	Doublewide/Extra Large	B136/B109
SE600/SE400 (18 x 16)	Jumbo/Enormo	B138/B111
SE640 (18 x 8)	Mini Doublewide	B136
SE660 (24 x 18)	Enormo/Colosso/2D Tray	B111/B140/B142
Ettan Dalt II/six/twelve (2D gel)	Colosso/2D Tray	B140/B142
Multiphor II, Excel 2D 12.5	Multi-Wide	B148
Multiphor II, Excel XL 12-14	Colosso/2D Tray	B140/B142
Invitrogen / Novex		
Novex XCell SureLock Mini	Mini Square	B144
Novex XCell SureLock Midi	Extra Large	B109
E-PAGE Gels	Extra Large	B109
Lonza (formerly Cambrex/FMC/BMA)		
PAGEr Gold Precast (8.3 x 7.1)	Medium	B101
PAGEr Gold Precast (8.3 x 8.1)	Mini Square	B144
Owl Systems		
Puffin P81	Large/Square	B107/B119
Penguin P8DS	Large/Square	B107/B119
Penguin P9DS	Jumbo/Enormo	B138/B111
Penguin P10DS	Enormo/Colosso/2D Tray	B111/B140/B142

Important Notes: 1) All above catalog numbers are given for the clear version. If black or colored versions are desired, check on catalog number. **2)** The above recommendations are not guaranteed to be correct. Please check the exact size of gels from your apparatus and compare it to the PerfectWestern® container (inside dimensions are given) to be sure.

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AP control	Negative control	Q701	50 mL	$453
AP control, in serum free media	Negative control	Q701-SFM	50 units	$453
Kit-AP	Stem Cell Factor	Q702	50 mL	$453
ELF2-AP	Elk, Cek5, Cek10	Q703	vial	$453
Sema3A-AP	Neuropilin	Q704	vial	$453
mP84-AP	IAP/CD47	Q705	vial	$453
IAP/CD47-AP	mP84	Q706	vial	$453
hIzumo-AP	Unknown	Q707	vial	$453
sTNFR2-AP	TNF-alpha, CD120	Q708	vial	$453
sCD4-AP	gp120, MHC class II molecules	Q709	vial	$453
IL-24-AP (Human)	IL-20R1/IL-20R2 and	Q710	vial	$453
IL-24-AP (Rat)	IL-22R1/IL-20R2 Receptors	Q711	vial	$453
AP-Collagen (Human, C-terminal of alpha1, Type I)	Endo180	Q712	vial	$453
AP-Collagen (Human, C-terminal of alpha1, Type III)	Unknown	Q713	vial	$453
AP-Collagen (Rat, C-terminal of alpha1, Type I)	Endo180	Q714	vial	$453
AP-LRR2-hSlit1	Robo1 and Robo2 Receptors	Q715	vial	$453
AP-LRR2-hSlit2	Robo1 and Robo2 Receptors	Q716	vial	$453

Same receptors for both constructs

AP-TAG® Reference List

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This list has just the first 61 of at least 212 References using our AP-TAG technology. For the complete list, go to: http://www.genhunter.com/support/aptag_references/