



**Rabbit (polyclonal)  
Anti- $\kappa$ -Opioid Receptor Antibody,  
Internal  
Unconjugated**

**PRODUCT ANALYSIS SHEET**

<b>Catalog Number:</b>	44302G
<b>Lot Number:</b>	See product label
<b>Volume:</b>	100 $\mu$ L
<b>Form of Antibody:</b>	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$ ), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
<b>Preservative:</b>	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
<b>Purification:</b>	Purified from rabbit serum by peptide affinity chromatography.
<b>Immunogen:</b>	The antiserum was produced against a chemically synthesized peptide derived from the internal region of the mouse $\kappa$ -opioid receptor. The sequence is conserved in rat.
<b>Target Summary:</b>	Activation of the $\kappa$ -opioid receptor opposes a variety of $\mu$ -opioid receptor mediated actions throughout the brain and spinal cord. In general, opioids modulate numerous central and peripheral processes, including pain perception, neuroendocrine secretion and the immune response. The opioid signal is transduced from receptors through G proteins to various different effectors. Subsequent to G protein activation, several effectors are known to orchestrate the opioid signal. For example, activation of opioid receptors increases phosphatidylinositol turnover, activates $K^+$ channels and reduces adenylyl cyclase and $Ca^{++}$ channel activities.
<b>Reactivity:</b>	Mouse and rat (100% homologous) $\kappa$ -opioid receptor. Human (93% homologous) $\kappa$ -opioid receptor has not been tested, but is expected to react.
<b>Applications:</b>	This antibody has been used in Western blotting. Other applications may work but have not been tested.
<b>Suggested Working Dilutions:</b>	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The exact concentration is not determined for each lot; however, the typical range is 0.1-1.0 mg/mL. The optimal antibody concentration should be determined empirically for each specific application.
<b>Storage:</b>	Store at $-20^{\circ}C$ . We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at $-20^{\circ}C$ . For shipment or short-term storage (up to one week), $2-8^{\circ}C$ is sufficient.
<b>Expiration Date:</b>	Expires one year from date of receipt when stored as instructed.
<b>Positive Control:</b>	Rat brain extracts.
<b>Related Products:</b>	<b>Antibodies:</b> $\mu$ -Opioid receptor (internal), Cat. # 44308G
<b>References:</b>	Cichewicz, D.L., et al. (2001) Changes in opioid and cannabinoid receptor protein following short-term combination treatment with Delta9-tetrahydrocannabinoid and morphine. J. Pharmacol. Exp. Ther. 297(1):121-127. Pan, Z.Z. (1998) $\mu$ -Opposing actions of the kappa-opioid receptor. Trends Pharmacol. Sci. 19(3):94-98. Piros, E.T., et al. (1996) Functional analysis of cloned opioid receptors in transfected cell lines. Neurochem. Res. (21)11:1277-1285.

**This product is for research use only. Not for use in diagnostic procedures.**

[www.invitrogen.com](http://www.invitrogen.com)

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

PI44302G

(Rev 11/08) DCC-08-1089

**Important Licensing Information** - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, [www.invitrogen.com](http://www.invitrogen.com)). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

## Western Blotting Procedure

1. Lyse approximately  $10^7$  cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH<sub>2</sub>O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat F(ab')<sub>2</sub> anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

### Cell Lysis Buffer

#### Formulation:

10 mM Tris, pH 7.4  
100 mM NaCl  
1 mM EDTA  
1 mM EGTA  
1 mM NaF  
20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>  
2 mM Na<sub>3</sub>VO<sub>4</sub>  
0.1% SDS  
0.5% sodium deoxycholate  
1% Triton-X 100  
10% glycerol  
1 mM PMSF (made from a  
0.3 M stock in DMSO)  
or 1 mM AEBSF (water  
soluble version of PMSF)  
60 µg/mL aprotinin  
10 µg/mL leupeptin  
1 µg/mL pepstatin  
(alternatively, protease inhibitor cocktail such as  
Sigma Cat. # P2714 may be used)

### Transfer Buffer

#### Formulation:

2.4 gm Tris base  
14.2 gm glycine  
200 mL methanol  
Q.S. to 1 liter, then add  
1 mL 10% SDS.  
Cool to 4°C prior to use.

### Tris Buffered Saline

#### Formulation:

20 mM Tris-HCl, pH 7.4  
0.9% NaCl

### Blocking Buffer

#### Formulation:

100 mL Tris buffered saline  
5 gm Ig-free BSA  
0.1 mL Tween 20

**This product is for research use only. Not for use in diagnostic procedures.**

[www.invitrogen.com](http://www.invitrogen.com)

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

PI44302G

(Rev 11/08) DCC-08-1089

**Important Licensing Information** - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, [www.invitrogen.com](http://www.invitrogen.com)). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.