

Certificate of Analysis

Anti-G_{q/11}α, CT (rabbit polyclonal IgG) Catalog # 06-709 Lot #

Immunogen: KLH-conjugated, synthetic peptide (QLNLKEYNLV) corresponding to amino acids 350-359 of $G_{11}\alpha$ or $G_q\alpha$. This sequence is highly conserved among species.

Specificity: Specific for $G_q \alpha$ and $G_{11} \alpha$. Does not recognize other non-pertussis toxin-sensitive G proteins.

Species Cross-reactivity: Human, mouse, and probably based on sequence homology, all mammalian species.

Formulation: 50μ I of protein A purified rabbit IgG in PBS, pH 7.4. Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/ thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Quality Control Testing

Immunoblot Analysis: A 1:500-1:2000 dilution of this lot detected $G_q \alpha$ and $G_{11} \alpha$ in 20µg of rat brain microsomal preparation (Catalog # 12-144). A previous lot detected $G_q \alpha$ and $G_{11} \alpha$ in 20µg mouse brain microsomes and membranes and $G_{q'11}$ alpha in 10µg of mouse 3T3/NIH fibroblast soluble plasma membranes.

Additional Research Applications

Immunoprecipitation: This antibody has been reported to immunoprecipitate $G_{q}\alpha$ and $G_{11}\alpha$.

	Immunoblot Analysis :
97 -	Representative lot data.
	Rat brain microsomal preparation was
66 -	resolved by electrophoresis, transferred
	to nitrocellulose and probed with anti-
45	$G_{\alpha/11}\alpha$, CT (1:2000 dilution). Proteins
	were visualized using a goat anti-rabbit
	secondary antibody conjugated to HRP
31 -	and a chemi-luminescence detection
	system. Arrow indicates $G_{\alpha}\alpha$ and $G_{11}\alpha$
21 -	(42kDa).
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Application References:

- 1. Arthur, J.M., et al., Am. J. Physiol. 273: 129-135, 1997.
- 2. Gettys, T.W., et al., Anal. Biochem. 220: 82-91, 1994.

General References:

- 3. Cote, M., et al., Endocrinology 138: 3299-3307, 1997.
- 4. Blanc, E.M., *et al.*, <u>J. Neurochem.</u> **69:** 570-580, 1997.
- 5. Gong, M.C., et al., Mol. Bio. Cell 8: 279-286, 1997.
- 6. Kuhn, B., et al., Mol. Endocrinol. 10: 1697-1707, 1996.
- 7. Cuq, P., et al., Eur. J. Pharmacol. 315: 213-219, 1996.

Immunoblot Protocol

- 1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on microsome or membrane sample and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
- 2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 30 minutes at room temperature with constant agitation.
- Incubate the nitrocellulose with a 1:500-1:2000 dilution of anti-G_{q/11}α, CT, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
- 4. Wash the nitrocellulose twice with water.
- 5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
- 6. Wash the nitrocellulose twice with water.
- 7. Wash the nitrocellulose in PBS-0.05% Tween[®]-20 for 3-5 minutes.
- 8. Rinse the nitrocellulose in 4-5 changes of water.
- 9. Use detection method of choice (enhanced chemiluminescence was used).

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