

# Product No. P-5704 Lot 064H4847

#### Monoclonal Anti-Protein Kinase C

Mouse Ascites Fluid Clone MC5

Monoclonal Anti-Protein Kinase C (mouse IgG2a isotype) is derived from the MC5 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Purified bovine brain protein kinase C was used as the immunogen.<sup>1</sup> The isotype is determined using Sigma ImmunoType<sup>TM</sup> Kit (Sigma ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The product is provided as ascites fluid with 0.1% sodium azide (see MSDS)\* as a preservative.

#### **Specificity**

Monoclonal Anti-Protein Kinase C (clone MC5) recognizes an epitope located within the amino acid sequence 296-317, at the hinge region, close to or at the trypsin cleavage site of protein kinase C (PKC). The antibody reacts with the 80 kD polypeptide of PKC, applying the immunoblotting technique using bovine brain PKC, extracts of rat glioma and murine NIH 3T3 cell lines, and immunoprecipitation of rat glioma and human cell extracts (lysates). Binding of the antibody to purified PKC *in vitro*, blocks partial proteolysis by trypsin. Introduction of Fab fragment of the antibody into a rodent glioma cell line inhibits phorbol-ester-induced down-regulation of the kinase. Cross-reactivity has been observed with human, bovine, rat and mouse. Also, the product may be used for immunohistochemical staining.

## **Description**

Protein kinase C (PKC, 77-90 kD) is one of a family of homologous serine-threonine protein kinases, that play a key role in signal transduction, cellular regulation, tumor promotion and oncogenesis. PKC is a calcium-dependent and phospholipid-dependent enzyme that is activated *in vivo* by the lipid diacylglycerol, produced in response to a variety of hormones and growth factors. PKC consists of a single polypeptide chain, containing four conserved regions and five variable regions. Sequence information defines a putative domain structure for the enzyme which can be divided into an amino-terminal regulator and a carboxy-terminal catalytic domain joined by a hinge region. Proteolysis of purified native PKC by trypsin yields two major fragments, representing the regulatory and kinase domains of the enzyme, due to cleavage in the proposed hinge region between residue 292 and residue 317.<sup>4</sup> There is evidence that *in vivo* agonist-induced generation of a catalytic fragment of the enzyme occurs as well. The PKC family of isozymes can be subdivided into two major classes; conventional (C) isoforms ( $\alpha, \beta_1, \beta_2$  and  $\gamma$ ), which are Ca<sup>2+</sup> and phospholipid-dependent kinases, and novel (n) isoforms ( $\delta, \epsilon, \zeta, \eta$  and  $\Theta$ ) that are Ca<sup>2+</sup>-independent, phospholipid-stimulated kinases. Antibodies that react specifically with PKC are useful for the study of the specific activation requirements, subcellular distribution, substrate specificities, and variation in mode of action of these isoenzymes. They also allow the detection and localization of PKC in normal and malignant tissues.

#### Uses

Monoclonal Anti-Protein Kinase C may be used for the localization of protein kinase C using various immunochemical assays such as ELISA, immunoblot, dot blot, immunoprecipitation and immunohistochemistry.

**Titer** 1:500

Titer is determined by indirect immunoblotting using rat brain cytosol preparation.

In order to obtain optimum results it is recommended that each individual user determine their optimum working dilutions by titration assay.

## Storage

For continuous use, store at 0-5 °C. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

\*A material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

### References

- 1. Young, S., et al., Eur. J. Biochem., **173**, 247 (1988).
- 2. Kikkawa, U., et al., Ann. Rev. Biochem., **58**, 31 (1989).
- 3. Nishizuka, Y., Science, 233, 305 (1986).
- 4. Parker, P., et al., Science, 233, 853 (1986).
- 5. Kiley, S., et al., J. Biol. Chem., **266**, 23761 (1991).

Sigma warrants that its products conform to the information contained in this and other Sigma publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice of packing slip for additional terms and conditions of sale.

Issued 09/94.