# ProductInformation

**ANTI-PKA, RII Subunit (cAMP-Dependent Protein Kinase, RII Subunit)**
**Developed in Goat, IgG Fraction of Antiserum**

Product Number **P 2854**

## Product Description

Anti-PKA is developed in goat using recombinant RII subunits of mouse PKA (cAMP-dependent protein kinase), expressed in *E. coli* as immunogen. The antibody is purified using protein G chromatography.

Anti-PKA reacts specifically with the RIIα and β subunits of PKA (51/54 kD). It reacts with mouse, rat and human.

Anti-PKA may be used for the detection of PKA by various immunoassays including immunoblotting, immunocytochemistry and immunoprecipitation.

The action of many hormones is mediated by the generation of the intracellular second messenger cAMP. The predominant effect of cAMP is to activate a cAMP-dependent protein kinase (PKA). Four molecules of cAMP bind each dormant PKA holoenzyme, activating the kinase by releasing the catalytic (C) subunits from the regulatory (R) subunit-cAMP complex. The cellular location of PKA is dictated by the R subunit. Two classes of R subunit exist; RI and RII, which form the type I and type II PKA holoenzymes respectively. The RI isoforms (RIα and RIβ) are thought to be primarily cytoplasmic whereas a significant proportion of the RII isoforms (RIIα and RIIβ) are particulate and up to 75% of the cellular RII pool associates with the plasma membrane, cytoskeletal components and endoplasmic reticulum, secretory granules or nuclei.

## Reagents

The product is supplied as IgG fraction in 0.07 M tris-glycine buffer, pH 7.4, containing 30% glycerol.

Protein concentration is approximately 3.5 mg/ml by Bradford.

## Storage/Stability

Store at 0 °C to –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

## Procedure

*Immunofluorescence*

1. Plate approximately 200 µl of a cell suspension into each well of a slide. Incubate 24 hours in a 37 °C. $CO_2$ incubator.
2. Wash the cells 3 X for 5 min. with PBS. Do not shake cells.
3. Add fixative (ice-cold, 95% ethanol, 5% acetic acid) for 1 min. at room temperature.
4. Wash the cells with PBS, 2 X for 15 min. Do not shake cells.
5. Add 400 µl PBS containing 0.08% BSA and incubate 30 min. at room temperature.
6. Wash cells with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of Anti-PKA in PBS containing 0.08% BSA and incubate overnight at 4 °C.
8. Wash the cells 2 X with PBS for 5 min.
9. Incubate the cells with a 1:400 dilution of anti-goat IgG conjugated with FITC (Sigma Product No. F 7367) in PBS containing 1% BSA for 1 hr. at room temperature.
10. Wash the cells 3 X with PBS for 30 min.
11. Examine the cells under a fluorescent microscope.

## Product Profile

Working concentration is 10 µg/ml by immunoblotting using a human A431 cell lysate. Detection was done using anti-goat IgG conjugated to peroxidase and enhanced chemiluminescence.

Working concentration is at least 10 µg/ml by immunofluorescence using A431 cells fixed with 95% ethanol and 5% acetic acid.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

## References

Coghlan, et al., J. Biol. Chem., **269**, 7658 (1994).
Coghlan, et al., Science, **267**, 108 (1995).
Lester, et al., J. Biol. Chem., **271**, 9460 (1996).