

PKA Assay in VSMC,

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1. Treat VSMC as desired. Wash a 10 cm dish of VSMC twice with PBS, and once with homogenation buffer (50 mM beta glycerophosphate, pH7.4, 1.5 mM EGTA, 1 mM DTT).
2. Scrape cells in the same buffer and resuspend to a volume of 1 ml, sonicate briefly and centrifuge at 10,000 X G @ 4C to remove debris.
3. Supernatant (5-10 ul) is assayed for PKA activity in a buffer (30 ul final volume) containing 25 mM beta glycerophosphate pH 7.4, 1.25 mM EGTA, 10 mM MgCl₂ and 0.5 mM DTT. The samples are assayed plus (0.17 mM) or minus Kemptide (SIGMA). The amount of ³²P-ATP (3000 Ci/mmole) included in each assay is 2 uCi. To demonstrate that this activity is PKA, the samples are also assayed plus or minus PKI inhibitor peptide (15 uM) (Sigma).
4. Incubate for 15 min at 30C. The reaction is terminated by spotting 25 ul of the reaction mix on squares (2 x 2 cm) of P-81 paper (Whatman). The paper are washed 4-5 times in 300 ml of 150 mM phosphoric acid, once in ethanol and dried with a hair dryer.
5. The papers are counted in a scintillation counter with 200 ul of ecolume fluor (Fisher)
6. The key to this assay, when using cell lysates is that the amount of PKA activity is proportional to the amount of cAMP carried over into the supernatants. As a control, the samples can also be assayed plus or minus 5 uM cAMP in the assay to give your 100% value. This will give full activation of PKA in the lysate. We find this value to be similar to a brief treatment of VSMC with forskolin (10 uM, 10 min).

Treatment X

- a: no kemptide
- b: plus kemptide
- c: plus kemptide, no PKI
- d: plus kemptide, plus PKI
- e: plus Kemptide, no PKI, plus cAMP
- f: plus Kemptide, plus PKI, plus cAMP

Kemptide	Sigma K1127
PKA inhibitor fragment 6-22 amide	Sigma P6062
cAMP,	Sigma A4137
beta-glycerophosphate,	Sigma G6251