

Kinases

Kinases are the most intensively investigated enzyme targets in drug discovery. A prerequisite for drug hits with physiological relevance is producing kinases in a functional state. One difficulty in this respect is the labile nature of kinases which tend to lose their activity during the isolation process thus requiring a reliable and extremely mild purification process.

Axxima AG, one of IBA's customers, focuses on obtaining a library of the presently known kinases. The company has established the production of functional kinases - which could not be achieved by using *E. coli* or insect cells - by optimizing an adenoviral expression system in combination with human 293 cells (Figure 1). For the subsequent purification of the kinases Axxima switched from a multi-step ion exchange purification scheme to the one-step *Strep*-tag affinity purification protocol. By using *Strep*-tag Axxima was enabled to get functional kinases in a short time directly from the mammalian cell lysates (Figure 2). The *Strep*-tag isolated kinases proved to be at least as functional as the kinases purified by the time consuming ion exchange protocol (Figure 3).

The results presented here are an excerpt from the article published by Cotton et. al. 2003, NAR 31, e128.

Figure 1

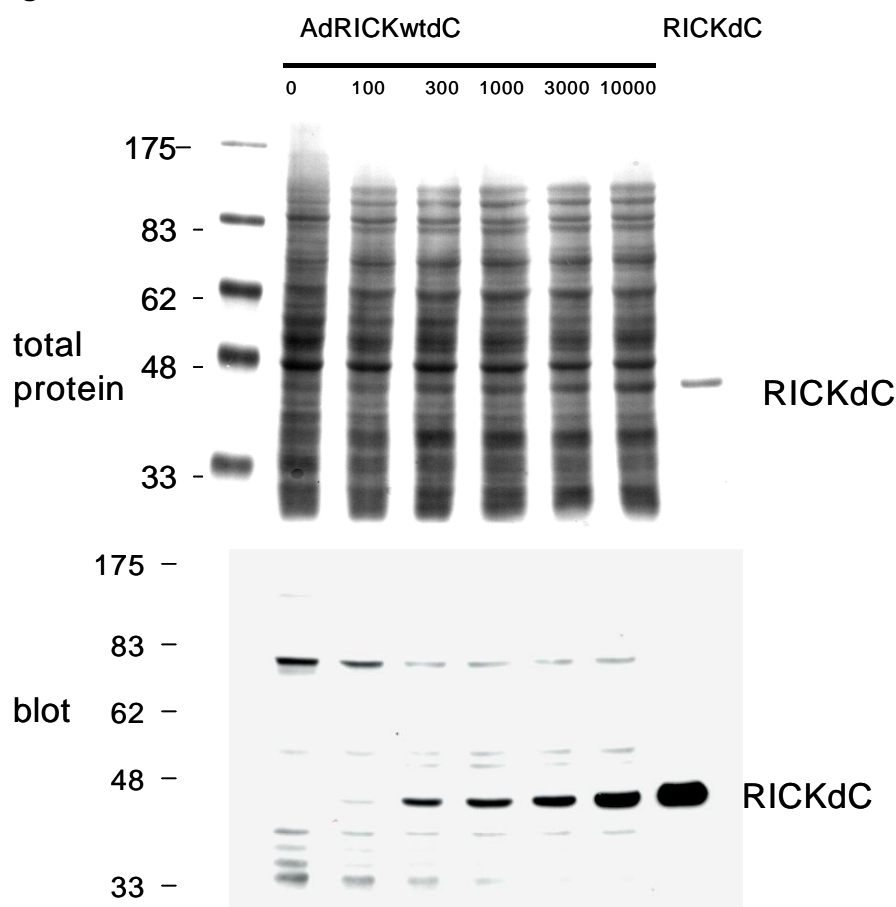
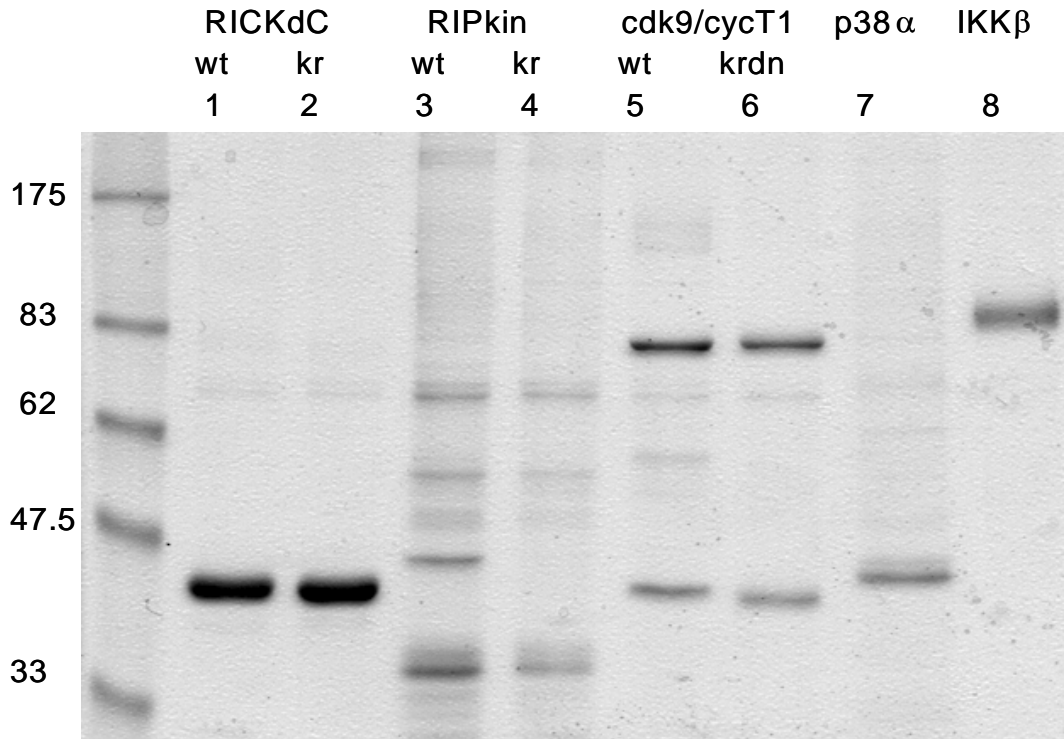


Figure 1: Optimization of infection conditions. Recombinant adenovirus directing the expression of the RICKdC were used to infect 293 cells at 100-10 000 virus particles/cell. At 48 h post-infection the cells were harvested, washed twice with HBS (150 mM NaCl, 20 mM HEPES, pH 7.5), lysed in SDS application buffer, heated at 99°C for 10 min, sonicated for 5 min in a sonicating water bath and 20 μ l were loaded per well on a 10% acrylamide SDS gel. After resolution, the gel was transferred to nitrocellulose, probed with *Strep*-Tactin-HRP (IBA) and visualized by ECL. Also resolved was purified RICKdC (last lane) and non-infected cell lysates (first lanes). Optimum recombinant kinase production is obtained at an adenoviral moi of 1000. Higher levels are obtained at an moi of 10000, however, the effort required to generate sufficient virus stocks becomes limiting at this moi.

Figure 2



coomassie stained gel

Figure 2: Equal aliquots of kinases purified by *Strep*-Tactin one-step affinity purification from 293 cells were resolved on a 10% acrylamide SDS gel and visualized by staining with colloidal Coomassie blue (Sigma). The contaminating proteins for RIPkin result from the co-purification of other specifically binding proteins forming a protein complex consisting of hsp90, cdc37, and the kinases cdk4 and raf1 and probably further kinases. This was investigated and demonstrated by Cotton et al. 2003.

Figure 3

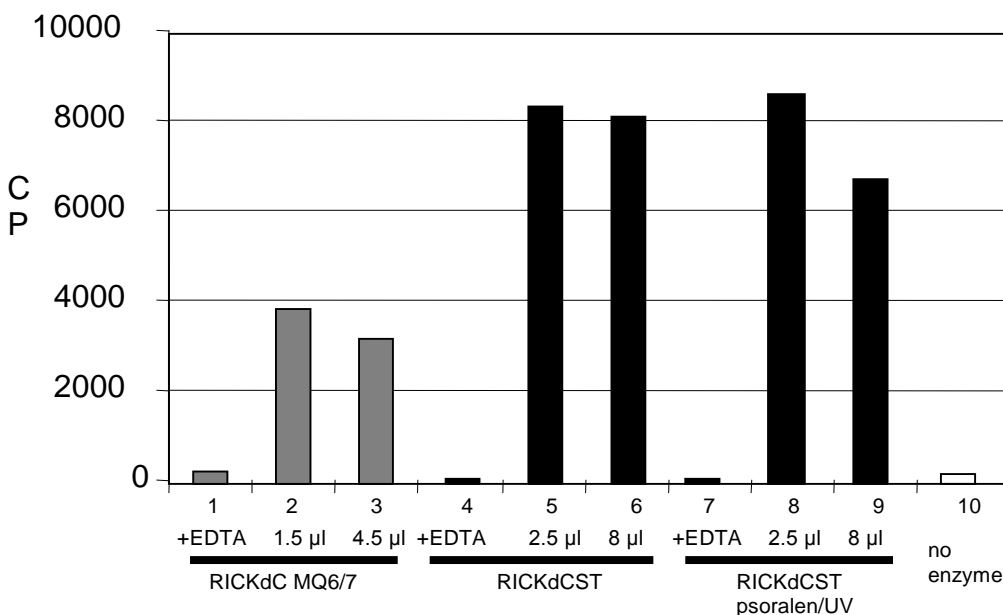


Figure 3: Samples were incubated with [γ - 33 P]ATP under kinase appropriate conditions and the radioactivity incorporated into a histone H2b substrate was measured. As can be seen the *Strep*-tag purified RICKdC kinase is as active as the same kinase without *Strep*-tag and purified via ion exchange chromatography.