

Usage tips for Affinity Material carrying immobilized ATP

1. ATP-Agaroses will bind ATP-binding proteins. The original reference characterizing these products is: Haystead *et al.* (1993) gamma-Phosphate-Linked ATP-Agarose for the affinity purification of protein kinases. *Eur. J. Biochem.* **214**:459.
2. AP-ATP-Agarose (AC-101) is immobilized via a phenyl moiety. This mimics a tyrosine residue, causing the material to be most suitable for tyrosine kinases (although serine/threonine kinases will also bind).
3. Blocking of the ATP-Agarose is important when purifying proteins from crude fractions where protein concentration is low, as this prevents non-specific binding to the Agarose matrix. A blocking method is described in the reference above. (For instance, pass crude cell extract over a column, and then regenerate the matrix with 10 mM ATP and high salt (0.5 M NaCl), followed by re-equilibration with several bead volumes of binding buffer).
4. Any protein with an accessible ATP-binding domain will bind to any ATP-Agarose, regardless of activation state. Although, activated proteins will bind with a higher affinity than the corresponding inactivated protein.
5. Proteins in which the ATP-binding domain is "hidden" in the inactive state, require activation to reveal the ATP-binding domain and enable binding to the Agarose.
6. Dehydrogenases can be purified using ATP-Agarose by omitting the NADH from the binding buffer, and using 10 mM NADH as the specific elutant.
7. Heat Shock Proteins (HSP) can be purified using ATP-Agarose by omitting ADP from the binding buffer and using 10 mM ADP as the specific elutant.
8. Better recovery and yields may be obtained when the ATP-Agarose purification is followed by ion exchange chromatography. This is reverse of how most researchers use the ATP-Agarose for purification.
9. β -Glycerol Phosphate is not recommended as a buffer component for the purification of kinases. Some users have noted that using this component has resulted in reduced activity and stability of some protein kinases.

Please contact info@jenabioscience.com with questions or inquiries.