Innova Biosciences

P_iBind™ resin

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1. INTRODUCTION

Inorganic phosphate (P_i) is a product of many enzymatic reactions, for example, those catalysed by phosphatases and ATPases. Measurement of released P_i allows enzyme activity to be determined, but contamination of enzymes or buffers by P_i can lead to unacceptably high assay backgrounds.

P_iBind™ resin provides a quick and easy way to remove contaminating P_i from buffers. The resin works over a broad range of pH values and is unaffected by many commonly used buffer additives.

P_iBind™ resin can also be used to remove P_i from protein samples (e.g. tissue extracts). This method is quicker than dialysis and unlike desalting does not necessarily lead to a significant dilution of the sample.

In most applications of P_iBind™ the resin is added to the contaminated sample or buffer solution which is then gently stirred or agitated. After adsorption of P_i, the resin is removed by either centrifugation or filtration.

Figure 1. Removal of P_i from 300ml of 0.1mM P_i solution by 1g of P_iBind™ resin

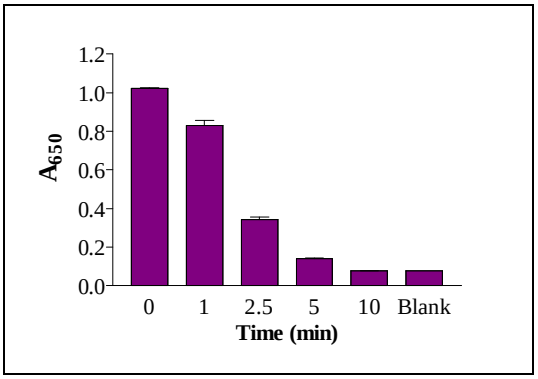

Figure 1 (above) shows that P_iBind™ resin efficiently removes P_i from a large volume of buffer. P_i was measured using P_iColorLock™ which is a modified malachite green reagent.

2. INSTRUCTIONS

2.1. Preparation of P_iBind™ resin

The resin is supplied as slurry in water. Upon prolonged storage or after rough handling (e.g. magnetic stirring) the solution above the resin may appear cloudy. In this situation, carefully decant the liquid and replace with distilled water. Since P_iBind™ is a dense matrix it settles rapidly, thus each wash step only takes a few minutes. This approach may also be used to exchange the resin into a buffer if required.

2.2. Pipetting of P_iBind™ resin

Many pipette tips have a fine bore at the point and pipetting of the resin may be difficult, particularly in the 50-200 µl range. This problem is easily overcome by using scissors to remove 3-4 mm of plastic from the end of the tip to increase the bore.

P_iBind™ resin settles quickly and it is necessary to agitate the solution while dispensing multiple aliquots. Magnetic stirring may be used with caution but gentler methods, such as shaking or overhead stirring, are less damaging to the beads. If any 'fines' are generated upon stirring the resin should be washed as described above in section 2.1.

2.3 Removal of P_i from buffers

To clear buffers completely of contaminating P_i the total capacity of the resin that is added to the buffer must exceed the amount of P_i in the solution. The capacity of P_iBind™ resin is >40µmol/g. In Figure 1, 300 ml of 0.1mM P_i, which contains 30 µmol of P_i, is cleared in 10 minutes with 1g of resin. The rate of clearance will be influenced by a number of factors (e.g. amount of resin, concentration of P_i, total amount of P_i, rate of mixing, and so on) and the best conditions in any given situation will have to be determined empirically. Certain buffer additives may reduce the rate of adsorption of P_i or the

capacity of P_iBind™ resin. Further information concerning interfering substances may be found in Table 1.

2.4 Removal of P_i from protein samples

2.4.1 Direct addition of resin

P_iBind™ resin is added directly to the sample and mixed by gentle inversion or by shaking on a rotary device. The resin may be separated from the clarified sample by filtration through filter paper or by centrifugation. With samples up to 2ml volume the resin may be pelleted by centrifugation in a microfuge for about 30 seconds, and the supernatant carefully removed with a pipette.

To minimise dilution of protein samples most of the liquid in a portion of P_iBind™ resin may be removed using a sintered glass funnel attached to a vacuum pump. It is necessary to apply suction for only a few seconds. Add the resin to the sample using a clean spatula.

2.4.2. Addition of resin to dialysis buffers

If protein samples are to be dialysed to remove phosphate, P_iBind™ resin can be added to the dialysis buffer to scavenge P_i. In this way it may be possible to reduce the number of buffer changes.

2.5. Compatibility with laboratory buffers

P_iBind™ resin has been evaluated using a variety of conditions and is effective over a broad range of pH values**.** Table 1 summarises the testing that has been carried out in our laboratories.

2.6. Storage of reagent

Upon receipt P_iBind™ should be stored at 4°C. Under these conditions excellent performance will be observed for a minimum of 6 months.

3. Ordering information

Product #501-0015 5 g (dry weight)

For larger quantities please contact our customer service department.

4. Related products

P_iColorLock™ is a family of modified Malachite Green reagents that can be used to measure P_i in a variety of different assay situations. For example, P_iColorLock™ ALS allows P_i to be measured in the presence of acid-labile substrates, such as ATP.

Further information on P_iColorLock™ products may be found on our web site: www.innovabiosciences.com.

Table 1. Buffer compatibility chart for P_iBind™ resin.

Conditions	Interference
100 mM Sodium Acetate, pH 5.0	None
100 mM MES, pH 6.0	None
100 mM MOPS pH 7.0	None
100 mM Hepes, pH 7.5	None
100 mM Tris pH 8.0	None
50 mM Glycine pH 2.3	None
100 mM Sodium Carbonate, pH 9.2	Significant
1-3 M NaCl	None
3 M KCl	None
100 mM Tris pH 8.0 / 0.1% Tween 20	None
100 mM Tris pH 8.0 / 0.1% bovine serum albumin	None
100 mM Tris pH 8.0 / 2mM dithiothreitol (DTT)	None
20% saturated ammonium sulphate/10 mM Tris, pH 8	Slight
50 mM Tris/150 mM KCl 500 mM imidazole, pH 7.5	None
100 mM Tris base	Significant
0.1 M HCl	Slight
100 mM MES pH 4.8	None
1M Hepes, pH 7.5	None
0.1 M HCl	None
5 mM EDTA, pH 8.0	Slight
50 mM Tris/ 150 mM NaCl/ 10 mM EDTA, pH 8.0	Slight

Note: Interference refers to an apparent reduction in capacity for P_i when the resin is incubated in the stated buffers. The results may also be explained by a reduced *rate* of clearance of P_i and we were not able to distinguish between these two possibilities under the experimental conditions employed.