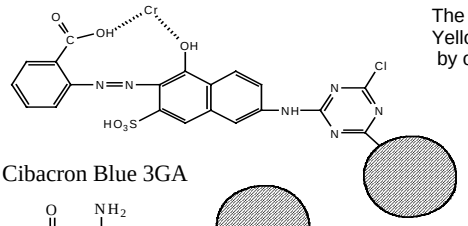
REACTIVE DYE AFFINITY CHROMATOGRAPHY MATRICES

ProductInformation

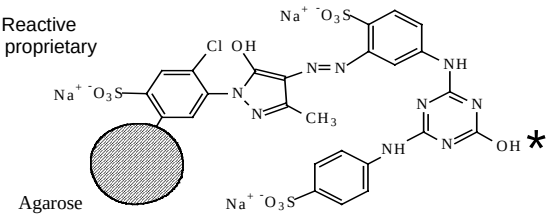
Reactive Dye Resins

Reactive Brown 10

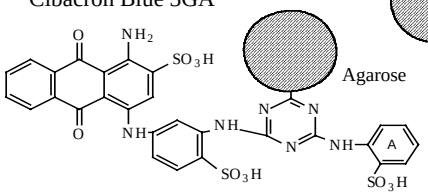


The structure for Reactive Yellow 13 is held proprietary by our supplier.

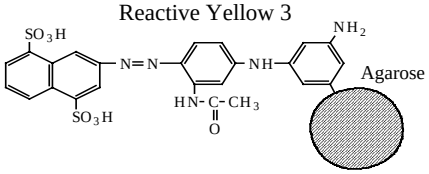
Reactive Yellow 2



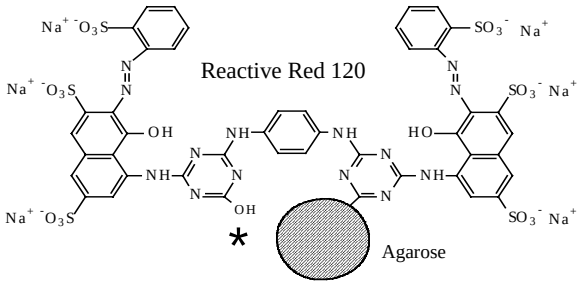
Cibacron Blue 3GA



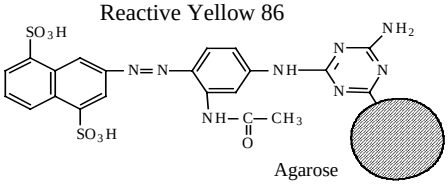
Reactive Yellow 3



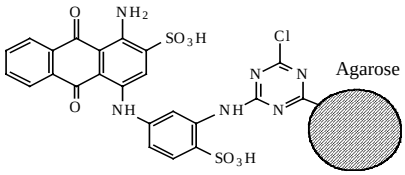
Reactive Red 120



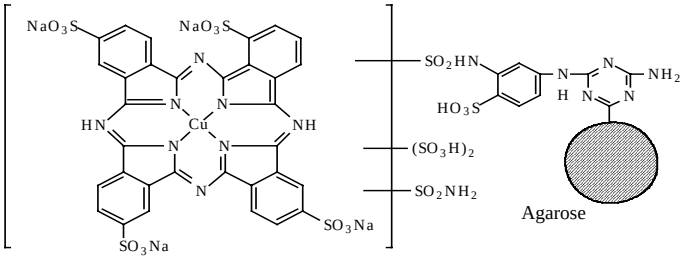
Reactive Yellow 86

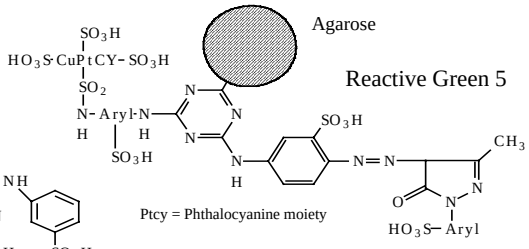


Reactive Blue 4

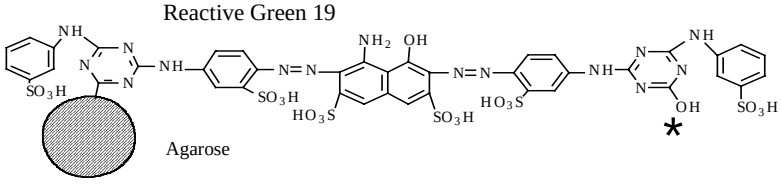


Reactive Blue 72





Reactive Green 19



* Alternate point of attachment of ligand to agarose

REACTIVE DYE AFFINITY CHROMATOGRAPHY MATRICES

GENERAL REMARKS:

Certain reactive textile dyes have been found to bind to proteins especially those with affinities to various nucleotides.[1,2] Immobilized dyes have been found to bind from 5 to 60 % of the proteins in various crude cell extracts.[3] The affinity of a specific dye for a particular nucleotide binding site on a protein cannot be predicted. The most effective method for the determination of a specific protein's binding capacity is a screening procedure with several types of immobilized reactive dyes.[3] (See Sigma Dye Resin Test Kit product number RDL-9). The affinity for reactive dyes to proteins may be due to substrate/cofactor similarities as well as hydrophobic and ion exchange properties. Some proteins have been found to require the addition of divalent cations for binding to dye resins.[4] Nonionic detergents have been found to encapsulate immobilized dyes in micelles and prevent proteins from binding. Low concentrations of anionic detergents will be repelled by the negative charge on most reactive dyes and may not interfere with protein binding.[5]

REFERENCES:

1. Kopperschlager, G., et al., *FEBS Lett.*, 1, 137 (1968).
2. Haeckel, R., et al., *Hoppe-Seyler's Z. Physiol. Chem.*, 349, 699 (1968).
3. Scopes, R.K., *J. Chromatog.*, 376, 131 (1986).
4. Hughes. P., et al., *Biochim. Biophys. Acta*, 700, 90 (1982).
5. Robinson, J.B., et al., *Proc. Natl. Acad. Sci.* USA, 77, 5847 (1980).

SUGGESTED GENERAL REFERENCES:

Stellwagen, E., *Meth. Enzymol.*, 182, 343 (1990).

Sigma offers several reactive dye affinity matrices. Matrices containing Cibacron Blue 3GA or Reactive Red 120 are available with varying amounts of dye bound. Higher dye content may lead to stronger binding of a given protein; lower dye content usually leads to more gentle elution conditions.

When properly regenerated and stored these matrices can be reused several times.

SUGGESTED CONDITIONS FOR USE:

Protein determination should be performed on the sample to be loaded as well as the wash and eluant fractions. Protein binding capacity will vary greatly and can exceed 20 mg per ml of resin. Most steps, with the exception of rehydration, can be done either in a column or batchwise using a filter funnel or centrifugation. Rehydration must be done batchwise. Care should be taken to prevent the media from drying out completely.

SUGGESTED CONDITIONS FOR USE: (continued)

1. a. Lyophilized matrices should be rehydrated with water or equilibration buffer using at least 200 mL/g. Rehydration should be done for a minimum of 30 minutes at room temperature or overnight refrigerated. The lactose used to stabilize the media during lyophilization should be washed out with water or equilibration buffer.

   b. Matrices in suspension should be washed with three to five column volumes of water or equilibration buffer.

   c. Equilibrate matrix with 5-10 column volumes of 0.01 M Tris HCl pH 7.5-8.0. Other buffer systems and additional components such as EDTA, divalent cations, or mercaptoethanol can be used. Some hydrolysis of the dye linkage may occur. Free dye must be washed out prior to usage.

2. Absorb the protein solution on the column.

3. Continue washing with 3-10 column volumes of equilibration buffer to remove unbound protein.

4. Elute with 0.01 M Tris-HCl pH 7.5-8.0 + 1.5 M NaCl. Other salts such as KCl, $CaCl_2$, NH_4Cl, or $(NH_4)_2SO_4$ may also be used. Other eluants may include pH shifts, nucleotides/cofactors (5-50 mM), Urea (0.5-6.0 M), guanidine, sodium thiocyanate, Triton X-100 (0.1-2%) or ethylene glycol (0.1-2%).

REGENERATION AND STORAGE:

Wash matrix with approximately 5-10 column volumes of each solution.

a. 0.1 M Borate pH 9.8 + 1.0 M NaCl (make at room temperature with boric acid and sodium hydroxide)

b. 0.1 M Borate pH 9.8 (alternate methods include high and low pH steps or chaotropic agents such as 6 M urea.)

c. Deionized water

d. 2.0 M NaCl (for storage)
Addition of an antimicrobial such as thimerosal or sodium azide should be used for long term storage. DO NOT FREEZE. Store at 2 - 8 °C.

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PRODUCT SPECIFIC BIBLIOGRAPHY:

Cibacron Blue 3GA

Cibacron blue 3GA has been shown to bind to several enzymes with known affinities to nucleotide cofactors. It has also been shown to bind to dehydrogenases[1], kinases[2,3], restriction endonucleases[4], albumin[5], and interferon[6]. There have been discrepancies in the exact structural differences between Cibacron Blue 3GA and Reactive Blue 2[7,8]. Our Cibacron blue 3GA preparations have the A-ring sulfonic acid group as shown in the illustration on page 1.

1. Lamkin, G.E. and King, E.E., *Biochem. Biophys. Res. Commun.*, 72, 560 (1976).
2. Thompson, S.T., et al., *Proc. Nat. Acad. Sci.* USA, 72, 669 (1975).
3. Kobayashi, R. and Fang, V.S., *Biochem. Biophys. Res. Commun.*, 69, 1080 (1976).
4. Baksi, K., et al., *Biochemistry*, 17, 4136 (1978).
5. Travis, J., et al., *Biochem. J.*, 157, 301 (1976).
6. Jankowski, W.J., et al., *Biochemistry*, 15, 5182 (1976).
7. Hanggi, D. and Carr, P., *Anal. Biochem.* 149, 91 (1985).
8. Burton, S.J., et al., *J. Chromatog.*, 435, 127 (1988).

Reactive Brown 10

Reactive Brown 10 has been shown to bind to Tyrosine-tRNA ligase.[1]

1. McArdell, E.C., et al., *Biochem. J.*, 243, 701 (1987).

Reactive Red 120

Immobilized Reactive Red 120 has been used for the isolation of NADP dependent dehydrogenases[1], and complement C9[2].

1. Watson, D.H., et al., *Biochem. J.*, 173, 591 (1978).
2. Eisenschenk, F.C., *Am. J. Vet. Res.*, 53, 435 (1992).

Additional information on Cibacron Blue 3GA (C1285) and Reactive Red 120 (R9129) matrices is available.