Product Information

# Reactive Dye Resins

**C1535, R2507, RB45, R0503, R3757**

Storage temperature 2-8 °C.

## Product Description

| Product Number | Product Description |
|---|---|
| C1535 | Cibacron Blue 3GA Agarose, Type 3000-CL, saline suspension |
| R2507 | Reactive Blue 4 - Agarosesaline suspension |
| RB45 | Reactive Blue 4 - Agarose, prepacked column |
| R0503 | Reactive Red 120 - Agarose saline suspension, Type 3000-CL |
| R3757 | Reactive Yellow 3 - Agarosesaline suspension |

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] The affinity for reactive dyes to proteins may be due to substrate/cofactor similarities (see structures in Appendix I) 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]

Several reactive dye affinity matrices are offered. Matrices containing Cibacron Blue 3GA are available with varying amounts of dye bound (see Appendix). Higher dye content may lead to stronger binding of a given protein; lower dye content usually leads to more gentle elution conditions.

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

Store at 2-8 °C. When properly cleaned and stored, these matrices can be reused several times.

## Procedure

See Appendix II for information regarding formulation of a purification scheme. 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 batch wise using a filter funnel or centrifugation. Rehydration must be done batch wise. Care should be taken to prevent the resin from drying out completely.

1. Rehydration and equilibration.
2. 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.
3. Matrices in suspension should be washed with three to five column volumes of water or equilibration buffer.
4. 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.
5. Absorb the protein solution on the column.
6. Continue washing with 3-10 column volumes of equilibration buffer to remove unbound protein.
7. 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%).

### Cleaning and Storage

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

1. M Borate pH 9.8 containing 1.0 M NaCl (make at room temperature with boric acid and sodium hydroxide)
2. M Borate pH 9.8 (alternate methods include high and low pH steps or chaotropic agents such as 6 M urea.)
3. Deionized water
4. 2.0 M NaCl (for storage)

Addition of an antimicrobial preservative should be used for long-term storage.

**DO NOT FREEZE.** Store at 2-8 °C.

## 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. Chromatogr., 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).
6. Stellwagen, E., Meth. Enzymol., 182, 343 (1990).


## Dye Specific Application Notes and References

### 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 Appendix I.


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. Chromatogr., 435, 127 (1988).


### Reactive Red 120

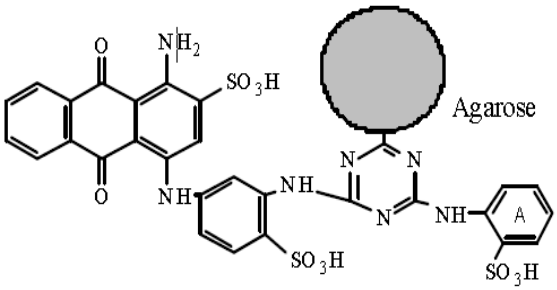
Immobilized Reactive Red 120 has been used for the isolation of NADP dependent dehydrogenases,[1] and complement C9.[2]


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

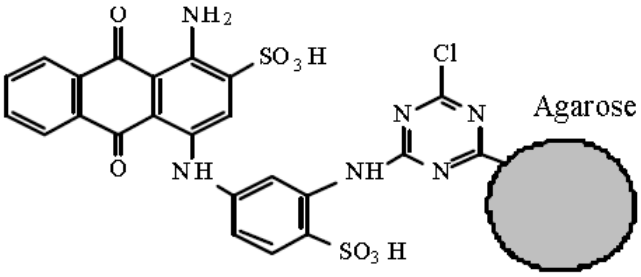
# Appendix I

## Reactive Dye Resin Structures

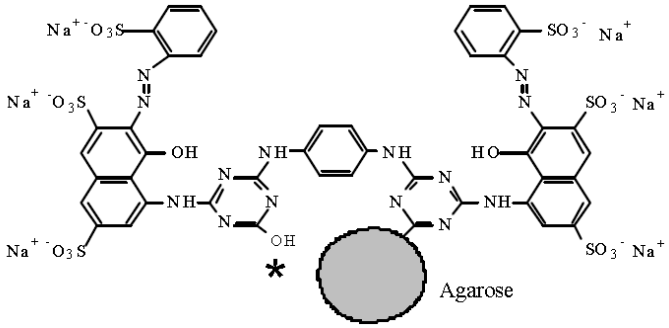
**Cibacron 3GA**



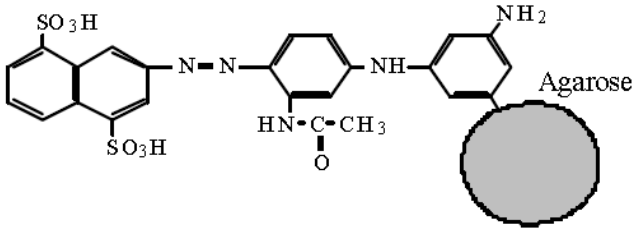

**Reactive Blue 4**




**Reactive Red 120**




**Reactive Yellow 3**




*Alternate point of attachment of ligand to agarose

# Appendix II

## Formulation of a purification scheme

Because of the non-specific nature of protein-dye interactions, the formulation of a purification scheme should be designed so that several parameters are kept as constant as possible. These parameters include:

1. Buffer systems
   - 1.1. The pH and ionic strength of the equilibrated column and the protein solution should match as nearly as possible.
   - 1.2. The buffer-protein compatibility – buffers should be non-denaturing to protein function.
   - 1.3. The resin-buffer compatibility – buffers should be specific for the desired function of the resin (i.e., high ionic strength for hydrophobic interaction or low ionic strength for affinity interaction).
2. Protein concentration
   - 2.1. The load solutions should be a constant mg protein/mL resin (This point is particularly critical). In cases of very low protein concentrations and very low mg protein/mL resin ratios, irreversible binding can occur.
   - 2.2. In some applications, the consistent loading of units/mL resin may be more reproducible.
   - 2.3. Differences in specific activity of load solutions can substantially alter the resulting chromatography.
3. Procedure
   Temperature optimum may vary; the recommended temperature is 2-8 °C.
   - 3.1. Equilibrate the column or columns with 5-10 column volumes of the chosen buffer.

3.2. Load the protein solution (concentration should be 1-10 mg/mL) to the column. In comparing columns it is generally advisable to load equal quantities of protein to each column.
**Note**: Centrifuge the sample to remove particles prior to loading on the column. Minimizing the lipid or lipoprotein content will aid in resin cleaning and help extend the column life.

3.3. Wash the load into the column with 0.1 mL to 0.5 mL equilibration buffer.

3.4. Continue to wash the column with equilibration buffer (5-10 column volumes) until no protein leakage is observed.

An example using Cibacron Blue 3GA Agarose resins to determine the following:

- Maximum binding effectiveness for differing levels of dye substitutions
- Maximum recovery
- Ease of recovery
- Degree of purification

**Example**

A theoretical composite based on a variety of results from a diverse sample of proteins. Data actually obtained varies from protein to protein. The most effective resin can be determined by individual results. In this example resin 1000 is the most efficient resin based on % recovery and degree of purity.

| | **Resin 3000** | **Resin 1000** | **Resin 300** | **Resin 100** |
|---|---|---|---|---|
| **Extent of labeling** | 2-5 µmol/mL | 0.8-1.2 µmol/mL | 0.2-0.5 µmol/mL | 0.05-0.2 µmol/mL |
| **Column size** | 2.5 mL | 2.5 mL | 2.5 mL | 2.5 mL |
| | Load 3 mL at 10 mg/mL; 10 unit/mL<br>Specific activity = 1 unit/mg | | | |
| **Units bound** | 30 | 25 | 20 | 15 |
| **Units recovered** | 24 | 24 | 15 | 4.5 |
| **mg recovered** | 12 | 6 | 4.2 | 1.5 |
| **Purification factor** | 2X | 4X | 3.5X | 3X |

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