

The reaction mechanism involves two steps: The activation of the carboxyl groups, and displacement by the nucleophile (R-NH<sub>2</sub>), releasing the EDAC as a soluble urea derivative.

The carboxyl groups can be on the ligand or on the matrix. The activated carboxyl groups react with the amino groups in the reaction mixture. Figure 1 is an illustration of the EDAC coupling reaction for a ligand with a terminal carboxyl group to Affi-Gel 102 gel.

## Ordering Information

Catalog Number	Product Description
153-0840	<b>CM Bio-Gel A Gel</b> , 250 ml
153-2401	<b>Affi-Gel 102 Gel</b> , 50 ml
153-0990	<b>EDAC Coupling Reagent</b> , 5 g
737-1007	<b>Econo-Column® Chromatography Column</b> ,* 1 x 5 cm, 2
737-1012	<b>Econo-Column Chromatography Column</b> ,* 1 x 10 cm, 2
737-1022	<b>Econo-Column Chromatography Column</b> ,* 1 x 20 cm, 2

\* Other sizes are available. Refer to the current Bio-Rad catalog.



## Immobilization Procedure for Affi-Gel® 102 and CM Bio-Gel® A Gels

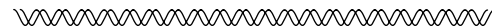
### Catalog Numbers

**153-2401**

**153-0840**



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LIT329 Rev B



## Immobilization Procedure

Assume a capacity of 15  $\mu$ moles/ml of available terminal groups.

1. Dilute the gel 1 to 1 with distilled water or appropriate solvent.\*
2. With gentle stirring (end over end, do not use a stir bar), add 10-50  $\mu$ moles of ligand/ml of gel (35-40 mg protein/ml gel) and adjust the pH to 4.7-5.0 with 1 N HCl.
3. Add 2-10 mg EDAC coupling reagent with continued stirring.
4. Immediately readjust the pH to 4.7-5.0 with 1 N HCl and let the reaction proceed for at least 3 hours.

Reaction pH, reaction time, concentration of EDAC coupling reagent, and coupling solvent\* can all be varied to optimize the degree of ligand binding.

\* The choice of coupling solvent is dependent on the solubility of the ligand. Coupling will occur under aqueous and anhydrous conditions. The following is a list of acceptable buffers.

Aqueous	Anhydrous
25 mm phosphate, pH 6.0	100% DMSO
25 mm MES, pH 6.0	100% isopropanol
50 mm acetate, pH 6.0	100% ethanol

For coupling under anhydrous conditions, the ligand must be in the free base form. This can be accomplished by precipitating the ligand from a basic solution.

## Coupling Chemistry

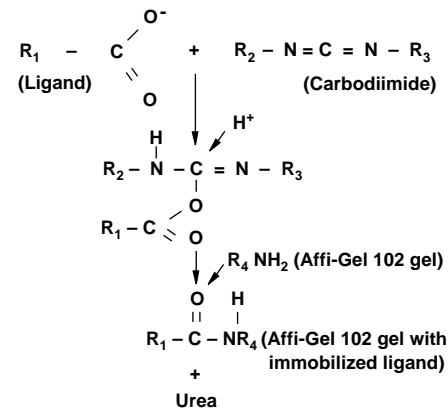


Fig. 1. EDAC coupling reaction.