



250PR-03

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A Geno Technology, Inc. (USA) brand name

SDC™ (Steroid/ Drug/ Compound) Immobilization Kit

Coupling Through Active Hydrogens
to Immobilized DADPA

(Cat. # 786-271)



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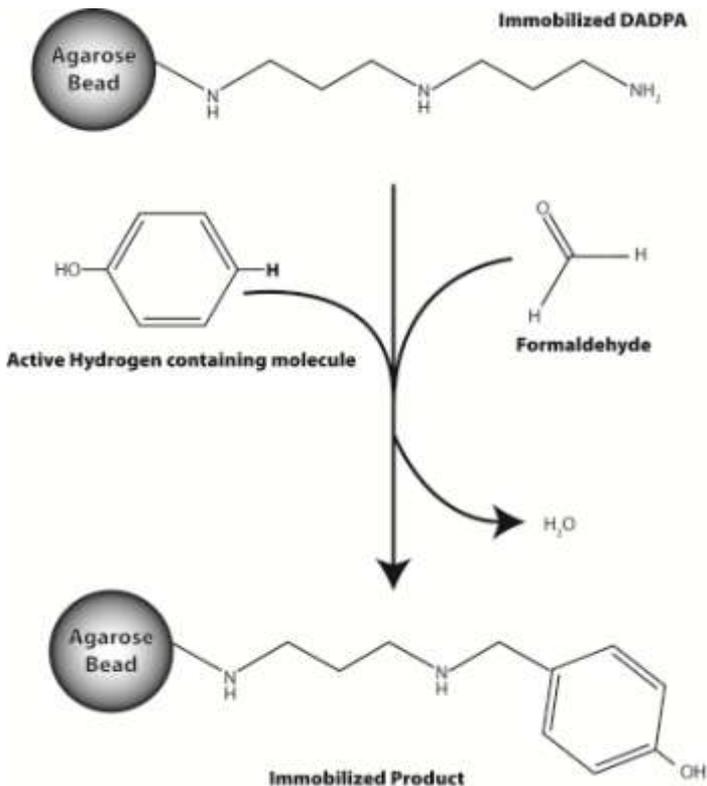
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INTRODUCTION

The SDC™ Immobilization kit is designed for the immobilization of steroids, drugs and chemical compounds that lack primary amines, sulfhydryls, carbonyls and other common coupling groups to a solid-phase agarose support for the use in affinity purification. The kit uses our Immobilized DADPA (diaminodipropylamine) resin that can bind steroids, drugs and chemical compounds through their active hydrogens.

The coupling uses the Mannich reaction, which is described as the condensation of formaldehyde with ammonia, in the form of its salt, and another compound containing an active hydrogen¹ (See appendix). The SDC™ Immobilization kit replaces the ammonia with the primary amine on the DADPA and the active hydrogen is supplied by the steroid, drug or chemical compound to be coupled. (see figure).



ITEM(S) SUPPLIED (Cat. # 786-271)

Description	Size
Immobilized DADPA (50% slurry)	10ml resin
SDC™ Coupling Buffer (0.1M MES, 0.15M NaCl, pH4.7)	60ml
SDC™ Coupling Reagent (37% Formaldehyde)	1.5ml
SDC™ Wash Buffer [10X] (1M Tris, pH8.0)	30ml
Spin Columns, 3ml	5
Caps	5
Rubber Stoppers	5

STORAGE CONDITIONS

Shipped at ambient temperature. Upon receipt store at 4°C, do NOT freeze.

SPECIFICATIONS

- **Activity:** ~60μmole amine/ml gel
- **Support:** 6% Cross-linked Agarose

ADDITIONAL COMPONENTS

- 37°C Waterbath
- 15ml conical tubes
- 2ml collection tubes

IMPORTANT

- Primary amines and formyl groups will interfere with the Mannich reaction. Avoid buffers, steroids, drugs and chemical compounds containing these groups.

PREPARATION BEFORE USE

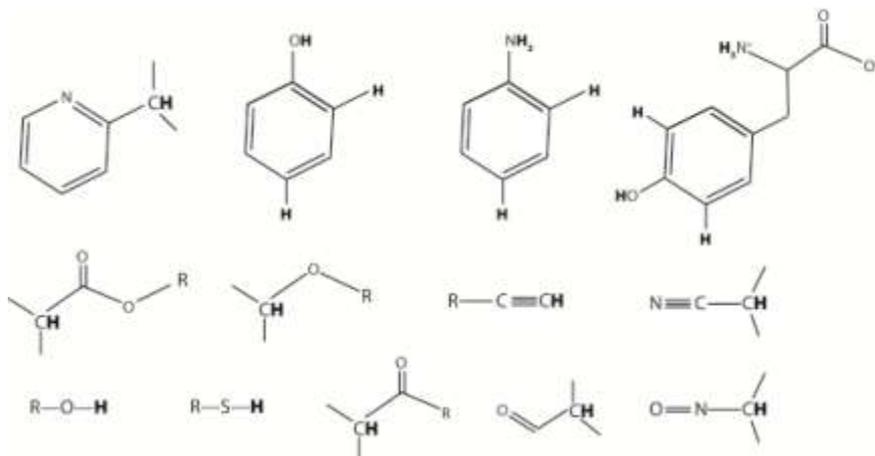
1. For every coupling experiment, add 6ml SDC™ Wash Buffer [10X] to 54ml deionized water to give a 1X solution.

PROTOCOL

Ligand Coupling

1. Allow the resin and reagents to warm to room temperature.
2. Transfer 4ml 50% Immobilized DADPA to a 15ml conical tube. Centrifuge at 700g for 2 minutes.
3. Carefully remove the storage buffer and discard.
4. Add 4ml SDC™ Coupling Buffer and gently mix to resuspend the resin.
5. Centrifuge at 700g for 2 minutes. And remove and discard the SDC™ Coupling Buffer. Repeat steps 4 and 5 once.
6. Dissolve the steroid, drug or chemical compound in 2-3ml SDC™ Coupling Buffer.
NOTE: For molecules that are not soluble in SDC™ Coupling Buffer, we recommend dissolving in a small amount of 100% ethanol and then combining with an equal volume of 50% ethanol/ SDC™ Coupling Buffer. If using ethanol, equilibrate the Immobilized DADPA resin with 50% ethanol/ SDC™ Coupling Buffer.
7. Add the steroid, drug or chemical compound solution to the resin.
8. Add 200µl SDC™ Coupling Reagent to the resin and seal the 15ml tube.
9. Incubate the resin slurry at 37-57°C for 3-24 hours with end-over-end mixing.
NOTE: The yield of conjugation is dependent on the reactivity of the active hydrogens and is hard to predict, therefore optimal immobilization needs to be empirically determined.
10. Following incubation, transfer the resin slurry to the supplied column. Use a small amount of SDC™ Wash Buffer to rinse any residual resin from the 15ml tube.
11. Allow the reaction buffer to drain from the column and then wash the column with 10 x 4ml SDC™ Wash Buffer.
NOTE: For insoluble molecules, wash the column with a 1:1 mix of SDC™ Wash Buffer and ethanol.
12. For long term storage, wash the column with 3 x 4ml SDC™ Wash Buffer supplemented with 0.02% sodium azide.
13. Seal the bottom of the column and ensure at least 1ml SDC™ Wash Buffer supplemented with 0.02% sodium azide is above the resin. Store upright at 4°C.

APPENDIX



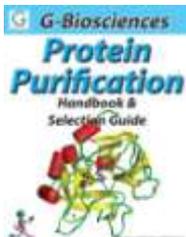
Examples of active hydrogen-containing compounds that can couple to resin through Mannich reaction.

REFERENCES

1. Hermanson, G.T. (1996) Bioconjugate Techniques. Academic Press, San Diego

RELATED PRODUCTS

Download our Protein Purification Handbook



<http://info.gbiosciences.com/complete-protein-purification-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.

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