

Operating Instructions

Affinity Chromatography Media (for ligand coupling)
Cellufine Formyl
Description

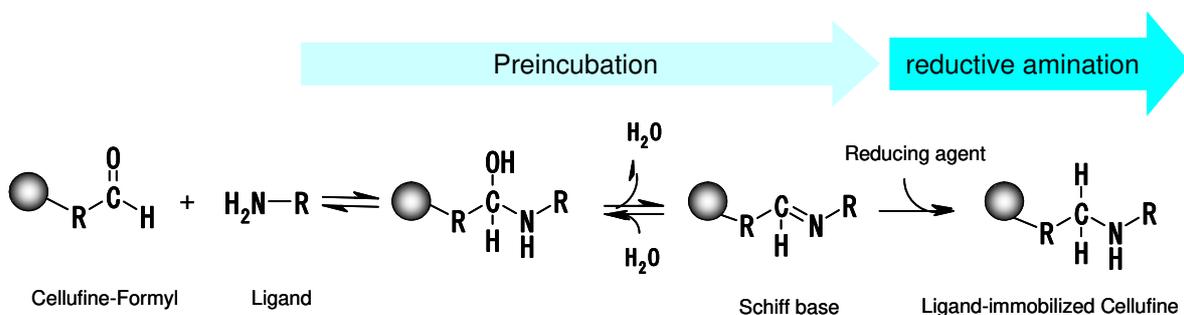
Cellufine Formyl is an aldehyde activated support for the covalent immobilization of amine containing proteins and ligands. As with all Cellufine products, the base support consists of spherical cellulose beads which exhibit superior rigidity and chemical stability relative to classical agarose gels. Such mechanical strength allows for improved throughput at both the bench scale and on the production floor. The exclusion properties of Cellufine Formyl are similar to those of 4 % agarose gels. Furthermore, due to the robust internal structure and chemical linkage, immobilized ligands show no appreciable leakage. With the use of a condensation agent, ligands (proteins, etc.) can be easily coupled via the reactive aldehyde moiety.

Physical-Chemical Characteristics

Base media	porous cellulose spherical beads
Particle Size	ca. 120-210 μm
Activated group	Formyl (aldehyde)
Formyl group conc.	10 $\mu\text{mol/ml-gel}$
Delivery condition	0.2M Acetate buffer,pH3.0 containing 0.01% 2,2-dithio-bis(pyridine-1-oxide)

Coupling (reductive amination)

The coupling of protein ligands having primary amino groups to Cellufine Formyl proceeds via a Schiff's-base intermediate followed by reduction with reducing agent NaCNBH_3 (SCBH), $(\text{CH}_3)_3\text{NBH}_3$ (TMAB) and NaBH_4 (SBH) as illustrated below:



Materials

- Coupling buffer: The buffer solution which does not contain a primary amine, the range of pH 4 to 11, and concentration are about 0.2M, a phosphate buffer, an acetate buffer, HEPES buffer solution, etc. can use.
- Blocking buffer: 0.2M to 1M of Primary-amine compounds in coupling buffer, such as ethanol-amine, glycine and glycine ethyl ester, etc or 0.2M to 1M of Tris-HCl buffer pH 7 to 8 is used.
- Reducing agent: the most common reducing agents are sodium cyanoborohydride (SCBH), Trimethylamjneborane(TMAB) and sodium Borohydride (SBH).

	Advantage	Demerit	Note	pH
SCBH (CAS 25895-60-7) (Sodjumcyanoborohydride) NaCNBH_3	Protein and aldehyde compound is not reduced, but a Schiff's-base is reduced selectively.	Toxic substance	used at a draft chamber Waste processing necessity	$\geq \text{pH}4$
TMAB (CAS 75-22-9) (Trimethylamjneborane) $(\text{CH}_3)_3\text{NBH}_3$	Reduction force is weak and there is almost no damage given to protein.	Solubility is low. (1.3% of solubility to water)	The pre incubation of 1 to 2 hour is required.	$\geq \text{pH}5$
SBH (CAS 16940-66-2) (Sodiumborohydride) NaBH_4	Blocking operation is unnecessary. Toxicity is low.	Since reduction force is strong, a formyl group is reduced. In special protein, use is impossible. (A S-S binding is reduced)	The pre incubation of 1 to 2 hour is required.	$\geq \text{pH}7$

Caution: Solution of SCBH should be made in a well ventilated area, since a small amount of toxic gas will be generated upon solubilization.

- Ligand solution: typically, 1 – 20 mg/ml in coupling buffer. Coupling efficiency decreases in 20mg/ml or more.

Note: total mass coupled will be directly related to concentration (see Fig. 1).

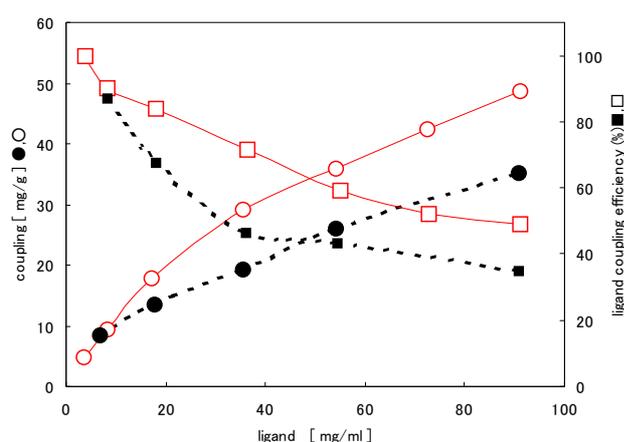


Fig.1 The effect of the concentration of ligand in the coupling reaction.

—○— h γ Glo. Coupling -●- HAS Coupling
—□— h γ Glo. Coupling ratio -■- HAS Coupling ratio

General Coupling Procedure

Table.1 The summary of operation (Required quantity per 1mL gel)

1	Cellufine Formyl	preservation solution is washing-removed. 1ml (0.7g to 0.8g- wet-g)
2	Coupling buffer	Coupling buffer solution containing ligand (1 to 2 ml) Refer to the "Coupling Considerations" for ligand concentration
3	Preincubation	agitates gently for 1 to 2 hours (preincubation is unnecessary when SCBH is used.)
4	Reducing agent	reducing agent adds 5-10mg. (Instead of powder, it's may add 50 to 100mg/ml of 1ml reducing agents.)
5	Coupling reaction	agitates gently for 2 - 8 hours It depends for the temperature suitable for a reaction on the stability of a ligand.
6	Washing	A reaction solution is removed by filtration or decantation. washes in several times by coupling buffer 20ml. The amount of coupling can be calculated by measuring the ligand concentration contained in reaction solution and washing solution.
7	Blocking reaction	1-2ml blocking solution is added to the washed gel, and the same quantity as a reducing agent (4) is applied. agitates gently for about 2 hours
8	Washing	A reaction solution is removed by filtration or decantation. Washes in several times by 20ml of coupling buffer 2 or water.

Perform the following in a suitable mixing vessel.

1. Estimate the required volume of medium. As for the bottle of a product, about 50% of Cellufine slurry is contained.
2. Preservation solution is removed by filtration or decantation. Washes in several times by coupling buffer 10 to 20ml. (until an acetic acid smell is lost.)
3. Add the ligand solution at a 1 to 2 times of gel volume. This will form a total slurry volume approximately 2 to 3 times the original volume of medium.
4. If using SCBH, preincubation is not necessarily required.
5. If using SBH, blocking reaction is unnecessary. Because, SBH is for reducing aldehyde (Formyl) and turning it a hydroxyl group.

Note

SCBH waste solution dispose of in a manner consistent with federal, state, and local regulations. And please refer to MSDS of a SCBH manufacture company.

Coupling Considerations

Ligand loading and biochemical activity are influenced by solute concentration, pH, reaction time and temperature. The coupling conditions above will be appropriate for most applications.

Ligand Concentration

Ligand loading is directly related to the ligand concentration. (See Fig.1.) Using a 50 % slurry (1 ml gel per 1 ml ligand solution) and about 10 mg/ml ligand concentration, 60 % coupling can be achieved in approximately 4 hours, at room temperature. Higher efficiency can be obtained

with longer reaction times.(See Fig.3)

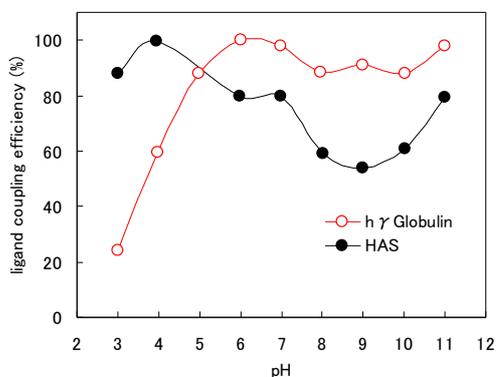


Fig.2 The effect of pH of coupling buffer on the amount of ligand coupled to the gel

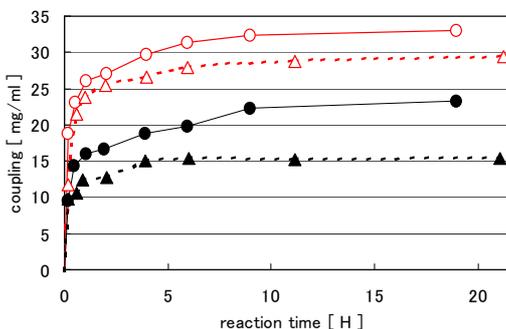


Fig3 The relation between the time for coupling reaction and the quantity of coupled protein.

—○— h γ Globulin(30°C) - -△- - h γ Globulin(4°C)
—●— HAS(30°C) - -▲- - HAS(4°C)

pH of a coupling buffer

Optimal pH changes with protein types.

pH stability and reaction efficiency of a ligand are taken into consideration.

Generally pH higher than pI goes up reaction efficiency .(See Fig.2.)

Schiff's base Formation and Reduction

A Schiff's base is readily formed between the amine ligand and aldehyde-gel. Sodium cyanoborohydride specifically reduces this linkage to a C - N bond, minimally affecting the ligand. Given such specificity, sodium cyanoborohydride can be added at time zero. In contrast, if BaBH₄ is used there should be a pre-incubation period in order to allow Schiff's base formation. Otherwise, the aldehyde moiety will be prematurely reduced.

Reaction Temperature

Lower temperature result in lower reaction rate and in some cases, less efficient coupling. As such, recommended reaction time should be increase to 16 hours, if refrigeration at 4 °C is required.

Column Packing

1. Calculate volume required for the desired bed dimension, keeping in mind that bed compression will occur during column packing.
2. Prepare a 40 – 60 % (v/v) slurry with the appropriate adsorption buffer.
3. With outlet closed, pour the slurry into column. Depending on the volume, a filler tube may be necessary.
4. With the inlet open to release air, insert and affix the top adjuster assembly at slurry

interface.

5. Open the column outlet and begin pumping adsorption buffer at rate at least 20 % higher than the operational flow rate.
6. After the bed stabilizes, close the column outlet. Then, with the inlet open, reposition the end cell on top of the bed.

Operating Guidelines

General Operation

1. Wash column with 5 bed volumes of elution buffer.
2. Equilibrate with 5 bed volumes of adsorption buffer.
3. Load sample.
4. Wash with 5 bed volumes of adsorption buffer.
5. Elute sample with 5 bed volumes of elution buffer

Recommended Buffers

These conditions will depend on the ligand used. However, the following are generally useful for immuno-affinity chromatography.

Adsorption buffer: 20 mM phosphate, 0.1 M NaCl (pH 7.2). Depending on application, other buffer ions may be used.

Elution buffer: 0.1 M glycine, pH 3.5

Sample Preparation and Load

Prepare a 1 – 10 mg/ml solution of sample in adsorption buffer. Remove insoluble material by centrifugation or microfiltration.

Flow Rate

The recommended linear velocity range for Cellufine Formyl is 20 – 150 cm/h.

Chemical and Physical Stability

The stability of the coupled gel will be limited by the ligand. However, the base gel is stable to most salts, detergents, chaotropic agents, 0.1 N NaOH, 0.1 N HCl and can be autoclaved at 121°C for 30 minutes at pH 7.0.

Regeneration

The regeneration protocol will depend on ligand stability. In some cases, a few bed volume washes with elution buffer containing 0.1 % Tween®20 or Triton® X-100 will be sufficient. A

similar wash with 6 M urea can also be effective.

Storage

For storage of opened containers, it is recommended that they be kept in a cold room (2 - 8 °C).
Do not freeze.

Shelf Life

3 years from date of manufacture

Product Ordering Information (Catalogue No.)

Media type	Pack Size				
	10 ml	50 ml	500 ml	5 lt	10 lt
Cellufine Formyl	676 944 324	19853	19854	19855	676 944 335

JNC CORPORATION

Chemical Division Life Chemicals Department
2-1, Otemachi 2-Chome, Chiyoda-ku, Tokyo 100-8105, Japan
Phone +81-3-3243-6150, Fax+81-3-3243-6219
E-mail: cellufine@jnc-corp.co.jp
<http://www.jnc-corp.co.jp/fine/en/cellufine>

TERMS AND CONDITIONS OF SALE

1. **Acceptance**-Buyer's placement of this order shall create a contract subject to and expressly limited by these terms and conditions. Acceptance may only be made on the exact terms and conditions hereof and if additional or different terms are proposed by Buyer, such response shall constitute a counteroffer. **THE TERMS OF THIS CONTRACT SHALL SUPERSEDE ANY CONFLICTING TERMS CONTAINED ON BUYERS PURCHASE ORDER OR ANY DOCUMENT OR INSTRUMENT SUBMITTED BY BUYER.**

2. **Prices, Taxes and Payment** - All prices are firm unless otherwise agreed to in writing. JNC Corporation reserves the right to change the prices and specifications of its Products at any time without notice. Any tax, duty, custom or other fee of any nature imposed upon this transaction by any federal, state or local governmental authority shall be paid by Buyer in addition to the price quoted or invoiced. In the event JNC Corporation is required to prepay any such tax, Buyer will reimburse JNC Corporation. Payment terms shall be net 30 days after shipment by JNC Corporation. An interest charge equal to 1½% per month (18% per year) will be added to invoices outstanding beyond 30 days after shipment. In addition JNC Corporation reserves the right to require C.O.D. payment terms from any Buyer whose account is overdue for a period of more than 60 days or who has an unsatisfactory credit or payment record. JNC Corporation may also refuse to sell to any person until overdue accounts are paid in full.

3. **Delivery and shipment** - JNC Corporation will make every effort to ship the Products or provide the services hereunder in accordance with the requested delivery date, provided, that JNC Corporation accepts no liability for any losses or for general, special or consequential damages arising out of delays in delivery. Shipment of all Products shall be F.O.B. point of distribution by JNC Corporation; identification of the Products shall occur when they leave JNC Corporation's point of distribution, at which time title and risk of loss shall pass to Buyer. All shipment costs shall be paid by Buyer and if prepaid by JNC Corporation the amount thereof shall be reimbursed to JNC Corporation.

4. **Inspection** - Buyer shall be responsible for inspecting all Products shipped hereunder prior to acceptance, provided, that if, Buyer shall not have given JNC Corporation written notice of rejection within 30 days following shipment to Buyer, the Products shall be deemed to have been accepted by Buyer.

5. **Disclaimer of Express and Implied Warranties** - The Products shall be covered by the applicable JNC Corporation standard warranty. **NO OTHER EXPRESS OR IMPLIED WARRANTY IS MADE WITH RESPECT TO THE PRODUCTS. JNC CORPORATION EXPRESSLY EXCLUDES THE IMPLIED WARRANTIES OF MERCHANTABILITY AND OF FITNESS FOR A PARTICULAR PURPOSE.** Any model or sample furnished to the Buyer is merely illustrative of the general type and quality of goods and does not represent that the Products will conform to the model or sample. Buyer's remedies under JNC Corporation's warranty shall be limited to repair or replacement of the Product or component which failed to conform to JNC Corporation's warranty. **JNC Corporation shall not be liable for any consequential damages or economic loss or property damage incurred by Buyer.**

6. **Returned Goods** - No Products shipped under this contract may be returned without the express prior authorization of JNC Corporation. All returns of Products are subject to a restocking charge. No returns will be authorized after 120 days following shipment to Buyer.

7. **Technical Advice** - JNC Corporation may, at Buyer's request, furnish technical assistance, advice and information with respect to the Products if and to the extent that such advice, assistance and information is conveniently available. It is expressly agreed that there is no obligation to provide such information which is provided without charge at the Buyer's risk and which **is provided subject to the disclaimers set forth in paragraph 5 above.**

8. **Agents, etc.** - No agent, employee or other representative has the right to modify or expand JNC Corporation's standard warranty applicable to the Products or to make any representations as to the Products other than those set forth in JNC Corporation's product literature and any such affirmation, representation or warranty, if made, should not be relied upon by Buyer and shall not form a part of this contract.

9. **Fair Labor Standards** - JNC Corporation represents that the Products or services provided hereunder were produced and/or performed in compliance with the requirements of all sections of the Fair Labor Standard Act of 1938, as amended.

10. **Equal Employment Opportunity** - JNC Corporation is an Equal Opportunity Employer. It does not discriminate in any phase of the employment process against any person because of race, color, creed religion, national origin, sex, age, veteran or handicapped status. The JNC Corporation Equal Opportunity Certificate, which is mailed annually to all vendors and vendees, is incorporated into this contract by reference.

11. **Modifications, Waiver, Termination** - This contract may be modified and any breach hereunder may be waived only by a writing signed by the party against whom enforcement thereof is sought.

12. **Governing Law** - This contract shall be governed by and construed in accordance with the laws (other than those relating to conflict of laws questions) of the Commonwealth of Japan.

13. **Arbitration** - Any and all disputes or controversies arising under, out of or in connection with this contract or the sale or performance of the Products shall be resolved by final and binding arbitration in Tokyo under the rules of the Japan Arbitration Association then obtaining. The arbitrators shall have no power to add to, subtract from or modify any of the terms or conditions of this contract. Any award rendered in such arbitration may be enforced by either party in either the courts of the Commonwealth of Japan District Court for the District of Japan, to whose jurisdiction for such purposes JNC Corporation and Buyer each hereby irrevocably consents and submits.