

CORNING® CELL-TAK™ CELL AND TISSUE ADHESIVE

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Lot No. _____

Instructions for Use

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WHAT IS CORNING® CELL-TAK™ ADHESIVE?

Corning Cell-Tak adhesive is a specially-formulated protein solution designed to be used as a coating on a substrate to immobilize cells or tissue. It can simplify the manipulation of biological samples in a number of common in vitro techniques, including:

- Establishment of primary cultures
- In situ hybridization
- Immunoassays
- Microinjection
- Immunohistochemistry

Corning Cell-Tak adhesive will readily coat a variety of materials, such as glass, plastics and even metals. The coating is transparent and stable for 10-14 days at 2-8°C.

Corning Cell-Tak adhesive is a formulation of the "polyphenolic proteins"¹ extracted from the marine mussel, Mytilus edulis. This family of related proteins is the key component of the glue secreted by the mussel to anchor itself to solid structures in its natural environment. The proteins are composed of tandemly repeated decapeptide units of similar amino acid sequence².

PRODUCT SPECIFICATIONS

FORMULATION: ____ mg/mL in 5% acetic acid

PACKAGING: Polypropylene vial containing 1 mg or 5 mg

STORAGE: Store at 2-8°C. Coated vessels can be stored for approximately two weeks at 2-8°C.

EXPIRATION DATE:

COATING METHODS

There are primarily two methods for coating a surface with Corning Cell-Tak adhesive: hand-spreading and adsorption. In general, we recommend adsorption because it is the more consistent and convenient method, but hand-spreading has its place for special situations.

HAND-SPREADING

The Corning Cell-Tak protein can be deposited on a glass or plastic surface by mechanical spreading. Using a handy tool, such as a glass rod or micropipette tip, microliter volumes of Corning Cell-Tak in 5% acetic acid can be spread in a thin liquid film. As the acetic acid evaporates, a coating of Corning Cell-Tak is left behind. After washing with ethanol and water, the vessels are ready to use.

Although hand-spreading gives a less uniform coating than adsorption it may be useful in special situations, for example when only a portion of a dish or glass slide is to be coated.

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ADSORPTION METHOD

The simplest and most cost-effective method of applying Corning® Cell-Tak™ is by adsorption from a neutral solution. The method is based on the observation that Corning Cell-Tak comes out of solution as the pH is raised and spontaneously adsorbs to the first surface it contacts. The resulting coating is quite thin (probably close to a protein monolayer) and more uniform than that achieved by hand-spreading.

The major advantages of this adsorption method are:

- coating vessels of any shape is simple
- less Corning Cell-Tak is required per cm² of surface area
- greater flexibility - the initial concentrations and adsorption times can be adjusted to best suit specific experimental protocols

Several precautions should be taken when using the adsorption method:

1. Adsorption begins immediately upon changing the pH; therefore, after diluting the Corning Cell-Tak into the neutral buffer, dispense within 10 minutes.
2. Since Corning Cell-Tak stores best in 5% acetic acid at 2-8°C, dilute the stock solution only when it will be used. If it is diluted into water, use the same day; if it is diluted into a neutral buffer, use immediately.
3. pH appears to be the most important variable regulating adsorption (a final pH between 6.5 - 8.0 is optimal). Although most buffers can be used to neutralize Corning Cell-Tak, sodium bicarbonate works best. If necessary, a volume of 1N NaOH equal to half the volume of Cell-Tak used may be added in combination with a neutral buffer to bring the solution to neutral pH.
4. Avoid introducing neutral buffer into the Corning Cell-Tak stock solution via a contaminated pipette or other means. Contamination of stock solution will result in precipitation of Corning Cell-Tak onto surface of stock vial with resultant loss of product.

BASIC ADSORPTION COATING PROTOCOL

READ THIS SECTION CAREFULLY BEFORE CONSULTING THE FOLLOWING PAGES FOR SPECIFIC DETAILS ON COATING PARTICULAR TYPES OF VESSELS OR DEVICES.

1. Prepare a neutral buffer solution. 0.1 M sodium bicarbonate, pH 8.0 is recommended when coating aseptically. Filter-sterilize the buffer.
2. Calculate amount of Corning Cell-Tak required. From the size and number of vessels to be coated, calculate total surface area. The best density of Corning Cell-Tak depends on your specific application, or cell type. A preliminary dose-response experiment is recommended to determine optimal density. Otherwise, start at a density of 3.5 µg Corning Cell-Tak/cm² of surface area. High densities will not necessarily improve performance, so the "minimum effective density" should be determined empirically.

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3. Neutralize the Corning® Cell-Tak™ and dispense to the vessel. The exact volume of buffer will depend on whether the sides of vessels will be coated. Dilute the correct amount of Corning Cell-Tak into the buffer, mix thoroughly, and dispense within 10 minutes.

NOTE: If the pH in the coating buffer is not between 6.5 - 8.0, Cell-Tak will not perform optimally. An aid to attaining this pH window is to use a volume of 1N NaOH equal to half the volume Cell-Tak solution used in combination with a neutral buffer. For example: Use 10 µl Cell-Tak, 285 µl Sodium Bicarbonate, pH 8.0 and 5 µl 1N NaOH (added immediately before coating) to make 300 µl Cell-Tak solution

4. Incubate for adsorption. A minimum incubation of twenty minutes is recommended, but longer times will not adversely affect adsorption, even if all the liquid evaporates. Pour off, or aspirate, the Corning Cell-Tak and wash with sterile water to remove bicarbonate. If vessels are to be used later, they should be air-dried and stored at 2-8°C up to two weeks or with desiccant up to 4 weeks.

SPECIFIC COATING METHODS

ADSORPTION METHOD FOR COATING TISSUE CULTURE DISHES, FLASKS, GLASS SLIDES AND COVERSLIPS

Points to consider:

- Coating aseptically, use the laminar flow hood for dilution and dispensing steps. For adsorption, vessels can be covered and moved to the bench top. Corning Cell-Tak adsorption occurs efficiently at both 37°C and room temperature.
- Speed up adsorption by minimizing volumes. Use only enough buffer to cover the bottom of the vessel. Check that the bottoms of the vessels are flat.
- Fill a flask or dish with buffer and then add Corning Cell-Tak directly. Remember to thoroughly mix the two liquids and change pipette tips before returning to the Corning Cell-Tak stock vial.
- Save reagent by basing calculations on effective growth areas, not on dish diameters. Some commonly used Falcon® plastic products and their effective growth areas follow:

Cell Culture Dishes	Effective Growth area/dish/well/flask
35mm	9.6 cm ²
60mm	20.0 cm ²
100mm	58.2 cm ²
Multiwell™ Cell Culture Plates	
6-well	9.6 cm ²
12-well	3.80 cm ²
24-well	2.00 cm ²
96-well	0.75 cm ²
Cell Culture Inserts	
6-well	4.2 cm ²
12-well	0.9 cm ²
24-well	0.3 cm ²
Cell Culture Flasks	
T-25	25 cm ²
T-75	75 cm ²
175 cm ²	175 cm ²
T-300	300 cm ²

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A. Coating Procedures - Flasks, dishes, coverslips and slides

1. Calculate the total surface area to be coated and the amount of Corning® Cell-Tak™ required.
2. Determine the volume of buffer needed to cover the bottom of the vessel.
3. For cell culture flasks, distribute the sterile buffer to each flask and add the appropriate amount of Corning Cell-Tak. Mix well. Do not cross contaminate Corning Cell-Tak stock with neutralizing buffer pipet tip, Corning Cell-Tak will adsorb to stock vial walls.
4. For cell culture dishes: mix the buffer and the Corning Cell-Tak in a tube, and then distribute the appropriate volume to each dish.
5. For coverslips or slides: The slides or coverslips can be laid on bottom of a culture dish and covered with the appropriate volume of neutralized Corning Cell-Tak solution. They can also be coated using the blood smear technique. Blood smear technique follows after this section.
6. Allow a minimum of 20 minutes for adsorption.
7. Pour off, or aspirate, the Corning Cell-Tak solution and rinse twice with sterile water.
8. If vessels are to be stored, air dry before storing at 2-8°C.

Blood Smear Technique (alternative method)

1. This method involves spreading a 20 microliter volume of neutralized Corning Cell-Tak across a standard microscope slide or coverslip.
2. Determine how many slides or coverslips are to be coated; make sure they are clean of dirt and grease. Start at a coating density of 20 ug Corning Cell-Tak per slide, or use an amount that has been previously determined for your application.
3. Transfer the appropriate volume of Corning Cell-Tak stock solution into a tube, and neutralize by adding one-fourth volume of **2M Sodium Carbonate**. Because of the small volumes used, we have optimized this technique by using 2M Sodium Carbonate in place of 0.1M Sodium Bicarbonate. The solutions will effervesce when mixed, so be sure to use a large enough tube. Adding 2-3% isopropyl alcohol to the stock solution will decrease surface tension and allow for complete coverage of glass slide or coverslip.
4. Slides can be coated by transferring 20 ul of neutralized Corning Cell-Tak to the center of a slide lying on a solid, flat surface. Spread the liquid evenly over the surface using the short edge of a second slide held at a 45° angle (this is the same technique used to make a blood smear). Coat the remaining slides in a similar manner.

Coverslips can be coated by placing a drop of the neutralized Corning Cell-Tak on coverslip, placing the second coverslip onto the drop of solution and allowing capillary action to spread solution between coverslips. Pull coverslips apart by sliding across each other.
5. Allow the slides or coverslips to air dry. The liquid will evaporate within five minutes at room temperature. Rinse the slides or coverslips with distilled water.

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6. If coated materials are to be used immediately, cells or tissue sections can be applied following the distilled water wash. Slides can be stored at 2-8°C, after air drying.

B. Coating Procedure for Multiple Well Plates

1. Calculate the total surface area to be coated and the amount of Corning® Cell-Tak™ required.
2. Determine the volume of buffer needed to cover the bottom of a well.
3. For plates with large wells, mix the total buffer volume and Corning Cell-Tak in a tube and dispense to the individual wells.
4. For small wells (96-and 48-well plates), place the appropriate amount of Corning Cell-Tak in a volume of 10 ul with water, into each well. Add a minimum of 20 ul of bicarbonate buffer to each well.
5. Allow at least 20 minutes for adsorption.
6. Flick, or aspirate off, the Corning Cell-Tak solution, and wash with distilled water.
7. If plates are to be stored, air-dry before storing at 2-8°C.

C. Coating Procedure for Permeable Supports (Cell Culture Inserts)

1. Calculate the total surface area to be coated and the amount of Corning Cell-Tak required. Since inserts are available in a variety of sizes, be sure to include the correct surface area in your calculations.
2. Determine the volume of buffer needed to fill the bottom of the insert.
3. Mix the total volume of buffer with the Corning Cell-Tak in a tube and dispense the appropriate volume to each device.
4. Allow at least 20 minutes for adsorption.
5. Aspirate the Corning Cell-Tak solution and wash with distilled water.
6. If the inserts are to be stored, air dry before storing at 2-8°C.

IMMOBILIZATION OF CELLS AND TISSUE

PROCEDURES FOR SEEDING CELLS

Both adherent and non-adherent cells stick when they come in contact with a Corning Cell-Tak coated surface. In fact, the cells need not even be viable. Fixed cells can also be immobilized, as well as microorganisms, such as yeast and bacteria.

Occasional problems with attachment have been traced to the fact that some cells (e.g., adipocytes, HL-60) and microorganisms do not readily settle out of suspension. In this case, reduce the total volume of medium in which the cells are seeded until it just covers the bottom of the vessel. If this does not improve performance cells might have to be centrifuged onto the surface.

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Cells may be seeded in serum-containing medium, but do **not** preincubate the medium in the Corning Cell-Tak -coated vessel before adding the cells. Serum proteins can block the adhesive sites. To increase cell attachment kinetics, try seeding cells in serum-free medium. Change to serum-containing medium immediately after cells attach.

We have observed that although non-adherent cells attach quickly to a Corning® Cell-Tak™ surface, they begin detaching within several hours. This detachment may be due to metabolic turnover of cell membrane proteins or to actual cell proliferation. The result is that non-adherent cells, such as hybridomas, will not form discrete colonies when grown on Corning Cell-Tak.

ADHERING TISSUE SECTIONS

Use standard techniques to pick up cryostat or paraffin tissue sections on a Corning Cell-Tak -coated slide. After mounting the section, remove excess moisture with a paper towel and transfer the slide to a 45°C warm table for at least one hour. Process the slide as required by the specific protocol.

REFERENCES

1. Waite, J.H. and M.L. Tanzer (1981) Science, **212**:1038-1040.
2. Waite, J.H. (1983) Journal of Biological Chemistry, **258**:2911-2915.

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