# Application of Corning® CellSTACK®-5 Chamber with the Corning CellBIND® Surface to Simplify and Enhance Mass-Production of Recombinant Proteins

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## Customer Application Note

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### Introduction

Extensive research on adhesion molecules in recent years has revealed that cell adhesion molecules are not just simple glue that mediates intercellular and cell-matrix interactions. They also function as adhesion-activated signaling receptors that convert extracellular stimuli into intracellular signals, so-called outside-in signals, as exemplified by cadherins and integrins. A single membrane-spanning glycoprotein, CADM1, is an immunoglobulin-like cell adhesion molecule originally identified as a tumor suppressor in non-small cell lung cancer. On the cell membrane plane, CADM1 forms homodimers (Figure 1). These homodimers then mediate intercellular adhesion through homophilic or heterophilic trans-interaction between neighboring epithelial cells. Although the short CADM1 cytoplasmic tail consisting of 42 amino acids lacks its own enzymatic activity, the protein 4.1-binding motif (protein 4.1-BM) and/or PDZ-binding motif (PDZ-BM) that reside in the cytoplasmic domain are believed to recruit adaptor molecules and/or signaling proteins required for its tumor suppresser function. So far, Dal-1, a member of the protein 4.1 family, and membrane-associated guanylate kinases (MAGuKs) have been found to interact with the cytoplasmic domain of CADM1 (Figure 1). It remains elusive, however, as to whether CADM1 transmits outside-in signaling.

### Materials and Methods

In order to examine whether CADM1 functions as an adhesion-activated signaling receptor, the CADM1-expressing cells were plated onto glass slides or cell culture plates coated with the ectodomain of CADM1 (Figure 2) in a planar substrate assay. This was followed by confocal microscopy and biochemical analyses of the activation of the signaling molecules. In performing this assay, an ample amount of CADM1 ectodomain was prepared as a recombinant protein tagged with the Fc portion of human IgG (ectoCADM1-Fc), enabling purification of ectoCADM1-Fc with protein A (Figure 2). Detailed procedures are shown in Figure 3. Briefly, human embryonic kidney (HEK) 293 cells were transfected with the ectoCADM1-Fc expression vector and ectoCADM1-Fc secreted into the serum-free media was collected twice after transfection. EctoCADM1-Fc was isolated from approximately 1.2L of the collected media using protein A agarose.

HEK293 cells were chosen to generate ectoCADM1-Fc for two specific reasons: 1) they are mammalian cells that give the CADM1 ectodomain proper N-linked and O-linked glycosylation, and 2) they are highly transfectable. Use of HEK293 cells, however, also has a definite disadvantage. These cells have weak adherence to conventional plastic cell culture dishes, especially in a serum-free medium

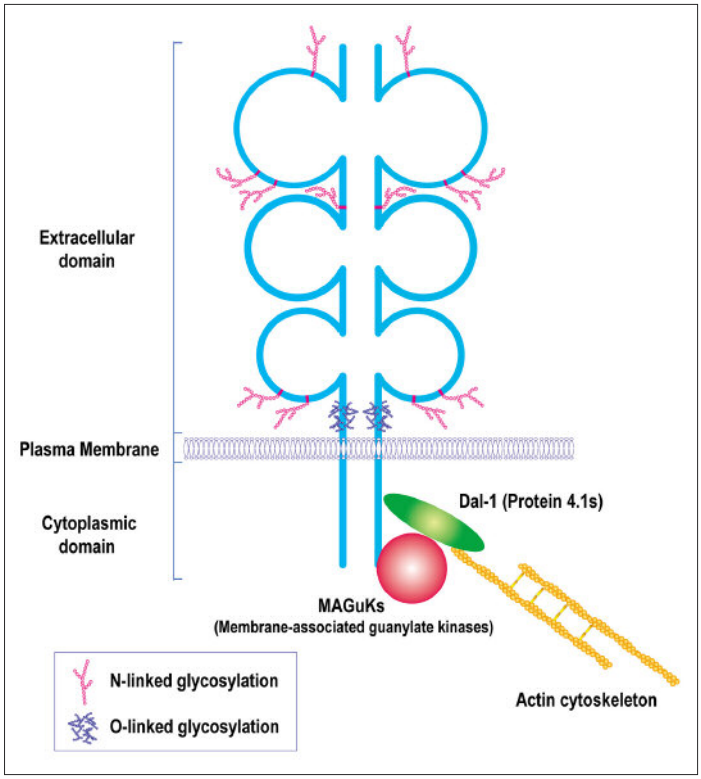


**Figure 1.** Structure of CADM1

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which is needed to avoid contamination with abundant serum-derived proteins. In addition, cells damaged by transfection come off the plates quite easily. Even successfully transfected cells cannot be sustained for long on the culture plates. Use of collagen I-coated plates can circumvent this problem; however, residual collagen in the purified recombinant protein is a concern in the subsequent assay. Fortunately, this problem was overcome by using the Corning® CellSTACK®-5 chamber with the Corning CellBIND® Surface (Corning Cat. No. 3311).

## Results

HEK293 cells adhered to the Corning CellBIND Surface much faster than to the plastic surface of conventional cell culture flasks. Transfection could be performed on the same day cells were seeded, resulting in saving one full day during the complete procedure (Figure 3). The yield of ectoCADM1-Fc was increased approximately three-fold compared with conventional plastic cell culture flasks, presumably due to the superior adherence and growth of HEK293 cells on the Corning CellBIND Surface (Figure 4). One preparation with this vessel produced enough of the ectoCADM1-Fc for repeated planer substrate assays.

In a planar substrate assay using ectoCADM1-Fc, the homophilic trans-interaction of CADM1 induced formation

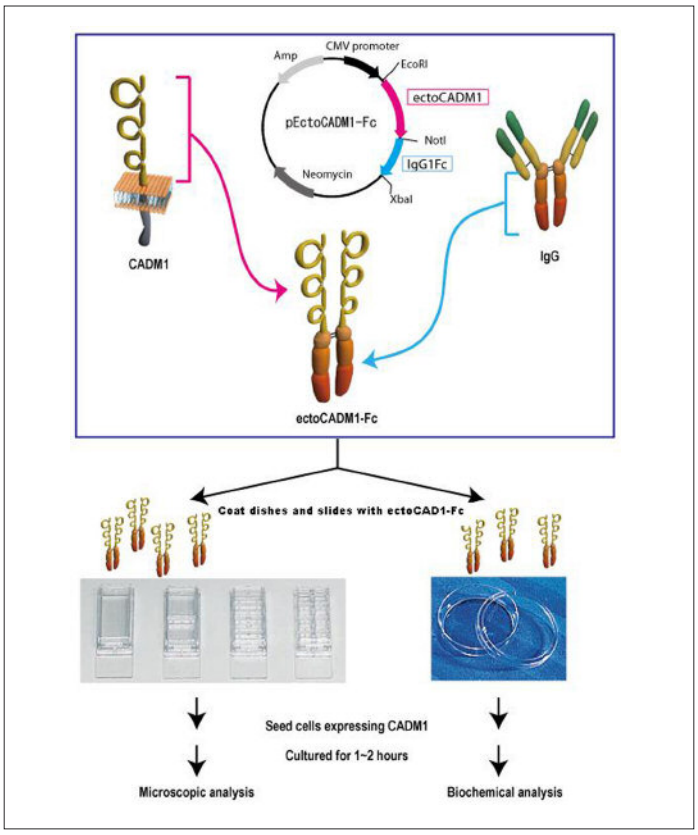



**Figure 2.** Planar Substrate Assay.

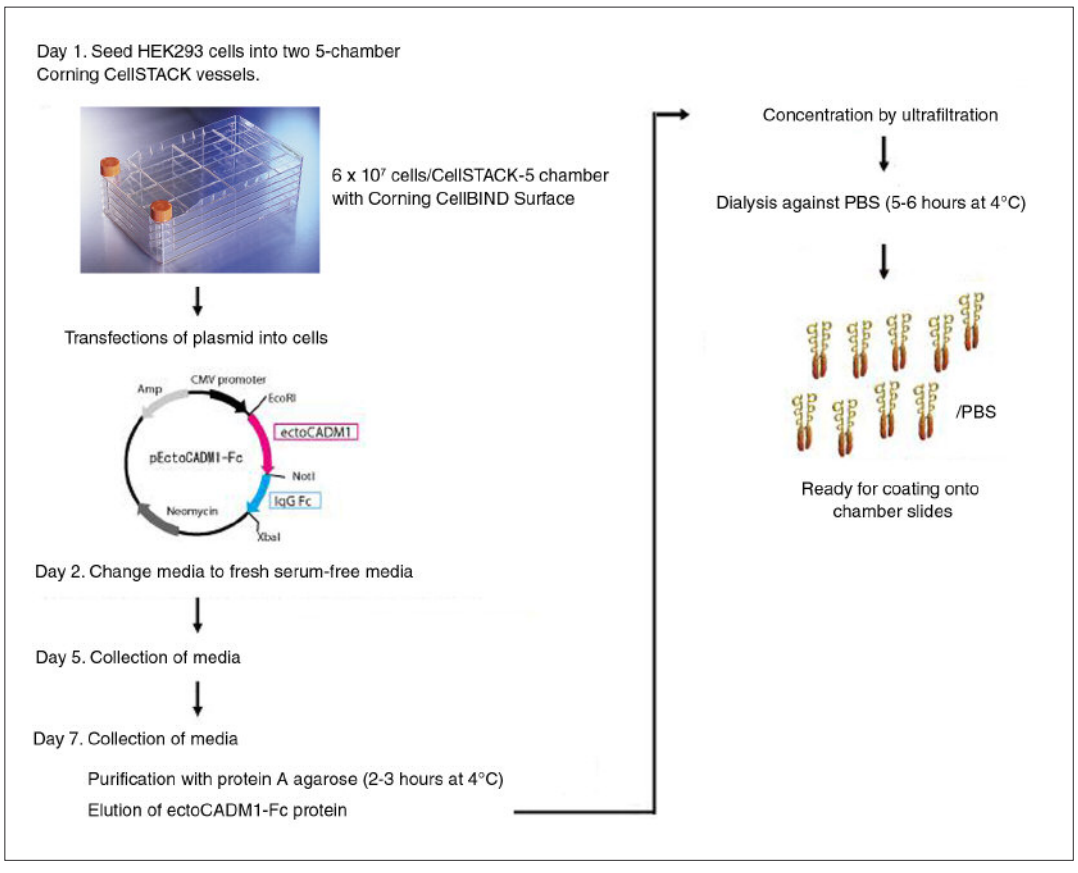



**Figure 3.** Procedure for producing recombinant protein using a CellSTACK-5 chamber with Corning CellBIND Surface. Due to better cell attachment to the Corning CellBIND Surface, transfection was able to be performed 8 hours after cell seeding. When flasks with traditional cell culture surfaces were used, cells needed to be plated one day before transfection.

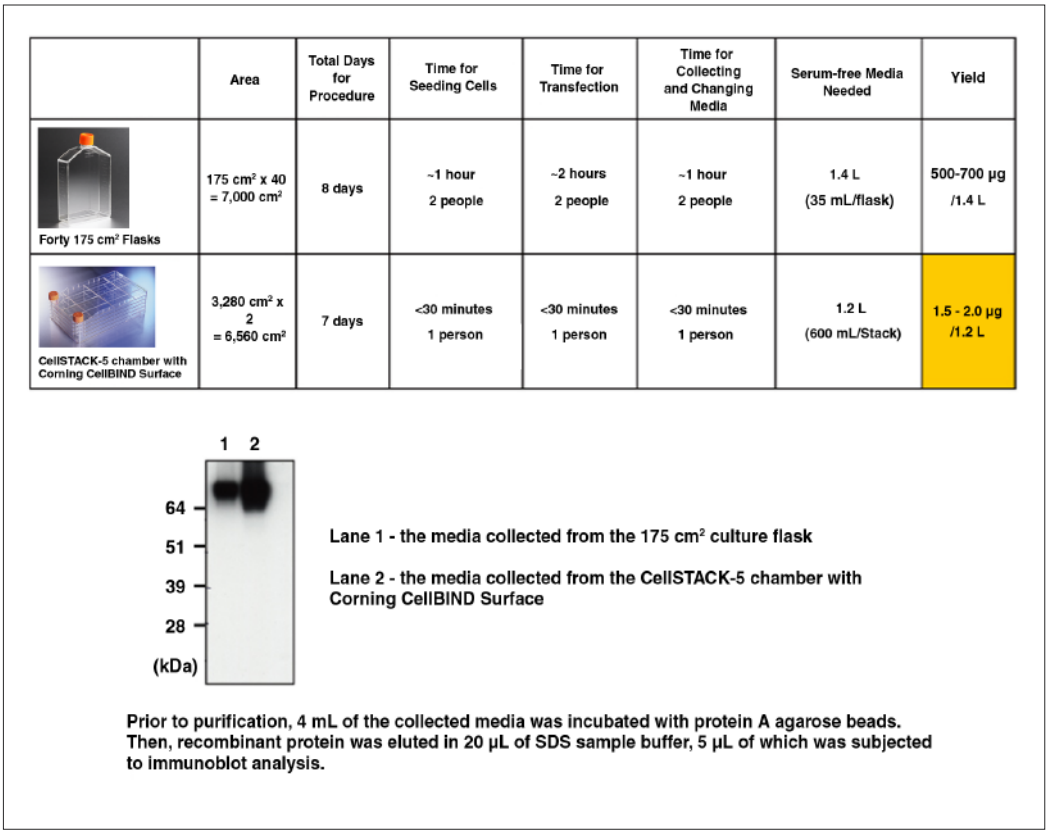
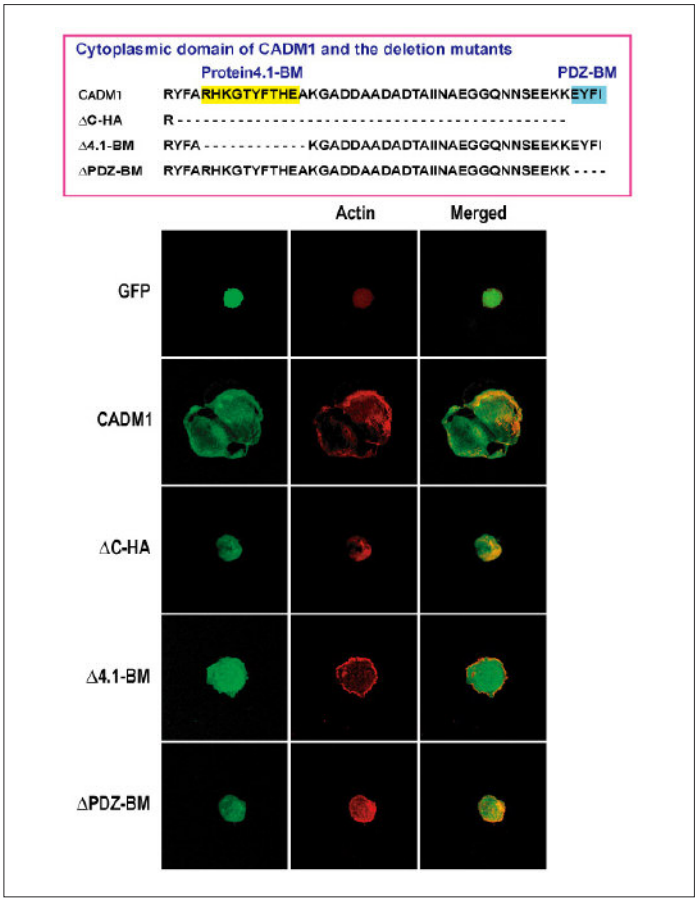



**Figure 4.** Advantage of Using CellSTACK®-5 Chamber with Corning® CellBIND® Surface for Production of Recombinant Protein. Note that an approximate three- to four-fold increase was observed with use of the CellSTACK-5 Chamber with Corning CellBIND Surface.




of lamellipodia in Madin-Darby canine kidney (MDCK) cells overexpressing CADM1. The lamellipodia formation was inhibited by a dominant negative form of a small Rho family GTPase Rac, indicating CADM1-induced formation of lamellipodia was Rac-dependent. Further analyses using deletion mutants lacking either the protein 4.1-binding BM or PDZ-BM revealed that the PDZ-BM was required for CADM-1 induced lamellipodia formation (Figure 5). These results indicated that CADM1 is indeed an adhesion-activated signaling receptor that transduces homophilic-ligation outside the cells to inside the cells leading to rearrangement of the cytoskeleton.

**Figure 5.** Trans-interaction of CADM1 Induces Lamellipodia Formation. Representative results of a planar substrate assay for homophilic trans-interaction of CADM1 are shown. Note that deletion of the PDZ-BM affects lamellipodia formation, indicating that the motif is necessary for this effect. GFP was expressed as a control. CADM1 and the deletion mutants (green) were immunostained with an antibody against the ectodomain of CADM1.

## Conclusions

It is becoming increasingly clear from accumulating published studies in recent years that CADM1 is a multi-functional adhesion molecule. It has even been reported to play important roles in the both the immune and neuronal systems. Depending on where CADM1 is expressed, it may activate cell signaling pathways unique to a particular tissue, thereby exerting biological functions specific to that tissue. The extracellular domain of CADM1 is also known to heterophilically interact with other adhesion molecules. The planar substrate assay using various recombinant proteins may provide answers to the molecular mechanisms of CADM1 signaling involved in pathogenesis of cancer and other diseases. Use of recombinant protein in investigating cellular signaling by adhesion receptors is highly versatile. During labor-intensive, time-consuming and costly procedures to generate recombinant protein, considerable effort, time and expense was saved by using the Corning® CellSTACK®-5 chamber with the Corning CellBIND® Surface as indicated in Figure 4.

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