Isolation and characterization of chimeric human Fc-expressing proteins using protein A membrane adsorbers and a streamlined workflow

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Abstract

Laboratory scale to industrial scale purification of biomolecules from cell culture supernatants and lysed cell solutions can be accomplished using affinity chromatography. While affinity chromatography using porous protein A agarose beads packed in columns is arguably the most common method of laboratory scale isolation of antibodies and recombinant proteins expressing Fc fragments of IgG, it can be a time consuming and expensive process. Time and financial

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constraints are especially daunting in small basic science labs that must recover hundreds of micrograms to milligram quantities of protein from dilute solutions, yet lack access to high pressure liquid delivery systems and/or personnel with expertise in bioseparations. Moreover, product quantification and characterization may also excessively lengthen processing time over several workdays and inflate expenses (consumables, wages, etc.). Therefore, a fast, inexpensive, yet effective protocol is needed for laboratory scale isolation and characterization of antibodies and other proteins possessing an Fc fragment. To this end, we have devised a protocol that can be completed by limited-experience technical staff in less than 9 hours (roughly one workday) and as quickly as 4 hours, as opposed to traditional methods that demand 20+ work hours. Most required equipment is readily available in standard biomedical science, biochemistry, and (bio)chemical engineering labs, and all reagents are commercially available. To demonstrate this protocol, representative results are presented in which chimeric murine galectin-1 fused to human Fc (Gal-1hFc) from cell culture supernatant was isolated using a protein A membrane adsorber. Purified Gal-1hFc was quantified using an expedited Western blotting analysis procedure and characterized using flow cytometry. The streamlined workflow can be modified for other Fc-expressing proteins, such as antibodies, and/or altered to incorporate alternative quantification and characterization methods.

Keywords

affinity chromatography; membrane adsorber; bioseparations; protein A; galectin-1; Gal-1hFc

INTRODUCTION

Isolation of antibodies and recombinant proteins expressing immunoglobulin G (IgG) Fc fragments from cell culture supernatants and dilute lysed cell solutions can be accomplished using protein A affinity chromatography. In basic science and engineering laboratories and industry, columns packed with porous protein A-coated agarose, glass, or polymeric beads are most commonly used for affinity chromatography, despite high financial costs and long processing times[1–3]. It is well-appreciated that affinity chromatography represents the largest expense and processing bottleneck in both lab and industrial settings[2,4]. As a result, numerous improvements have been developed to decrease cost and processing time without sacrificing overall product recovery from the process train[1,2,5–7]. Particularly promising for the isolation of antibodies and other Fc-expressing proteins is affinity chromatography using a protein A membrane adsorber[2,5,7–10]. Whereas Fc capture in column chromatography is diffusion-limited (i.e., the Fc-expressing protein must diffuse into and through internal pores to reach the majority of the protein A) with high pressure drop across the column, mass transport in membrane adsorbers is driven by bulk convection, resulting in avoidance of diffusion limitations[8,9,11,12]. In addition, pressure drop across the membrane adsorber is low, thereby permitting faster perfusion flow rates compared to bead-packed columns[8,9,11,12]. Thus, for laboratory scale isolation, membrane chromatography is predicted to reduce isolation time by several hours and increase product capture compared to column chromatography. Furthermore, mathematical models for IgG adsorption in membrane adsorbers have been proposed[8,9,11,12], thus allowing end-users to predict performance in the lab.

Another bottleneck in basic science and engineering laboratories is the characterization of the Fc-expressing protein. However, selection of appropriate methods to complete characterization assays, such Western blots, can greatly reduce time spent on product testing. For instance, semi-dry transfer in a discontinuous buffer system can accomplish electrophoretic transfer of proteins from an SDS-PAGE gel to a polyvinyl difluoridine

(PVDF) membrane in a matter of minutes, as opposed to 1–2 hours in tank (wet) transfer in a continuous buffer system[13].

A rapid, inexpensive, and effective protocol to isolate and characterize a chimeric fusion protein expressing an Fc fragment of human IgG is described herein. Most equipment is readily available in standard basic biomedical science, biology, chemistry, and (bio)chemical engineering labs, and all reagents are commercially available. Although representative results were generated from the isolation and characterization of a murine galectin-1/human Fc chimeric fusion protein (Gal-1hFc), the streamlined protocol can be applied to the isolation of other molecules that possess an Fc fragment, such as antibodies, Fc fragments themselves, or other Fc fusion proteins. Our improvements dramatically decreased processing time (as few as 4 hours but more typically ~9 hours, compared to 20+ work hours) while achieving similar or improved product recovery compared to standard methods.

PROTOCOL

1. Verify that the Fc-expressing protein (recombinant fusion protein or IgG antibody, heretofore referred to as "protein") has acceptable affinity for protein A[14–17] prior to using the protein A membrane adsorber, and test for the presence of the protein in the stock solution (e.g., cell culture supernatant clarified by centrifugation at $1000 \times g$ for 5 min to pellet suspended cells). Use flow cytometry, Western blotting, ELISA, etc., to detect protein function and/or presence of Fc, based on lab preference. Ideally, quantify the protein in the cell culture supernatant (step 6) to avoid exceeding protein A membrane adsorber capacity. Proceed if protein is detected.

2. Prepare protein A membrane adsorber and cell culture supernatant.

    2.1) Follow the manufacturer's instructions for preparing the membrane adsorber. Never let air enter the membrane adsorber. All solutions perfused through the membrane adsorber should be at room temperature and pre-filtered using a 0.22 µm filter. Fill a 10 ml syringe with 0.22 µm-filtered Dulbecco's phosphate buffered saline (DPBS) or buffer of choice, and discharge air bubbles. Perfuse DPBS to remove the storage solution and to equilibrate the membrane adsorber immediately before use.

    2.2) Filter the cell culture supernatant immediately before perfusion through the membrane adsorber, using a vacuum-driven 0.22 µm sterile filtration unit.

3. Load the protein A membrane adsorber.

    3.1) Aspirate cell culture supernatant into a Luer Lock syringe (30 ml or larger), and discharge any air bubbles.

    3.2) Assemble materials and equipment as shown in Figure 1. Connect the syringe to the inlet of the membrane adsorber. Attach flexible tubing to the membrane adsorber outlet. If desired, place a 0.22 µm syringe filter between the syringe and the membrane adsorber. Use a flask or bottle to catch flow through (also known as filtrate), from the adsorber.

    3.3) Set the syringe pump to the desired volumetric flow rate, but do not exceed the manufacturer's recommended flow rate (e.g., 10 ml/min). Perfuse the cell culture supernatant through the membrane adsorber,

collecting flow through in a beaker or other container. Reload the syringe with supernatant as needed.

3.4) If desired, test the flow through for the presence of protein using flow cytometry, Western blotting, ELISA, etc., based on lab preference. It is typically unnecessary to re-perfuse the flow through.

4. Elute protein from the membrane adsorber.

4.1) Wash the membrane adsorber with 10 ml DPBS to remove any non-bound protein.

4.2) Elute protein from the membrane adsorber at the desired flow rate (e.g., 1 ml/min) using 10–15 ml of elution buffer (e.g., amine-based elution buffer (pH 2.8)). Catch eluate in a tube containing neutralizing buffer (e.g., 1 M Tris (pH 9.4)) at 10% of elution volume (1.0–1.5 ml).

4.3) Alternatively, elute protein in one ml increments into tubes containing 100 µl of neutralization buffer, using a total volume of 10–15 ml elution buffer. Use the preferred characterization method to test each fraction for the presence of protein.

4.4) Regenerate the membrane adsorber according to the manufacturer's instructions. Perfuse 10 ml of 0.22 µm-filtered DPBS or buffer of choice, then 10 ml of 0.22 µm-filtered 50 mM NaOH in 1 N NaCl, and finally 10 ml of 0.22 µm-filtered DPBS. Fill the membrane adsorber with 20% ethanol in DPBS for long-term storage at 4°C.

5. Concentrate and dialyze the protein.

5.1) Deposit all eluate (or elution fractions containing protein as determined in step 4.3) in a 10 kDa molecular weight cut-off centrifugal filter unit. Follow the manufacturer's instructions for centrifugation.

5.2) Dialyze the retentate in a small volume dialysis unit (10 kDa molecular weight cut-off) against the buffer of choice, following the manufacturer's instructions. Buffer may need to be added to dialyzed material if protein precipitation is a concern. If desired, the dialyzed material can be refrigerated until step 6 can be performed, but long term storage without preservatives is not recommended.

6. Quantify and characterize purified product in an expedited Western blotting procedure.

6.1) Resolve purified protein and Fc standards on the gel of choice by SDS-PAGE, under reducing or non-reducing conditions as desired[18–20]. Use a mini gel rather than midi gel to minimize run time.

6.1.1) If not already known, determine the Fc standards range over which band signal varies linearly with respect to quantity of loaded (e.g., 0.1 µg to 1 µg). Use the same gel, running conditions, transfer conditions, and reagents that will be used to quantify and characterize purified protein.

6.1.2) Load multiple sample amounts of purified protein, including samples diluted with DPBS, to ensure that the purified protein band signals are within the linear signal range of Fc standards in image analysis.

6.2) Transfer proteins from the gel to a polyvinylidene fluoride (PVDF) membrane in a discontinuous buffer system using a semi-dry blotter[13], following the blotter manufacturer's instructions. Transfer time is typically 5–10 min. If desired, Coomassie stain the gel after transfer to verify transfer efficiency[21].

6.3) Perform immunoblotting with anti-Fc or anti-IgG conjugated to alkaline phosphatase (AP) or horse radish peroxidase (HRP), based on lab preference, using a vacuum-assisted protein detection system. Immunoblotting time is typically less than 1 hr.

6.4) Develop immunoblot using the substrate and method of choice (e.g., AP substrate and enhanced chemiluminecence).

6.5) Quantify purified protein by image analysis. Perform a linear regression on the band signals from Fc standards. If $R^2 > 0.90$, use the equation for the line to calculate protein quantity from its band signal(s). Account for sample dilution if necessary. Since quantification of the protein is performed on the basis of Fc, adjust the value for molecular weight differences between the protein and Fc. If $R^2 < 0.90$, repeat step 6 in its entirety.

7. Validate protein using functional assays or methods to detect Fc via flow cytometry, Western blotting, ELISA, etc., based on lab preference.

8. Dilute protein to desired concentration and/or add any desired preservatives or stabilizers to the purified protein solution before aliquoting for long-term storage, frozen or otherwise.

REPRESENTATIVE RESULTS

The streamlined protocol for isolation and characterization of Fc-expressing proteins is routinely used to process chimeric murine galectin-1 fused to human Fc (Gal-1hFc) from dilute cell culture supernatants. The flowchart in Figure 2 illustrates the workflow and time for each step in the protocol. For a typical batch of 300 ml of supernatant, the total processing time is approximately 9 hours when the optional flow cytometry testing of elution fractions is performed. If all flow cytometry analysis is omitted, due to lack of a flow cytometer or individual lab preference, total processing time can be as brief as 4 hours. In general, processing time depends on operator experience, amount of supernatant processed, and which quantification and characterization methods are performed.

Flow cytometry was used to test for the presence of functional Gal-1hFc (i.e., Gal-1 able to detect its ligands on breast cancer cells) in the starting cell culture supernatant; fresh cell culture medium served as the negative control[18,22]. Once Gal-1hFc in the cell culture supernatant was verified (Figure 3A), loading of the protein A membrane adsorber was accomplished by supernatant perfusion at 10 ml/min (resulting in superficial velocity or volumetric flux of 0.5 cm/min in a 2 ml membrane adsorber) using a syringe pump (Figure 1). The membrane adsorber efficiently captured and retained Gal-1hFc, leaving the flow through essentially depleted of Gal-1hFc (lack of breakthrough, Figure 3A). Gal-1hFc was eluted from the membrane adsorber in one milliliter increments at 1 ml/min (volumetric flux = 0.05 cm/min) using an amine-based acidic buffer (pH 2.8), and neutralized elution fractions were subsequently tested for the presence of functional Gal-1hFc using flow cytometry (Figure 3B). Gal-1hFc signal sequentially increased from elution 1 (approximately equal to negative control) to a maximum at elution 4, then decreased to a nearly constant level at elutions 9 and 10 (Figure 3B and 3C). The final elution 10 did not reach equivalence to the negative control signal due to some adsorber retention of Gal-1hFc

(Figure 3B and 3C). However, this loss was deemed acceptable. As an alternative method to test for presence, but not function, of Gal-1hFc in various solutions, flow cytometry analysis of Gal-1hFc's ability to bind to protein A polystyrene beads (through recognition of the hFc region) can be used (Figure 3D).

Following elution fraction testing, Gal-1hFc-positive elutions 2 through 10 were concentrated and then dialyzed against DPBS to obtain purified Gal-1hFc in the desired buffer. Quantification and characterization of the purified Gal-1hFc was performed in a Western blotting procedure expedited by semi-dry transfer and vacuum-assisted immunoblotting. By comparing Gal-1hFc signal to hFc quantification standards, both the quantity and quality of purified material were determined (Figure 4A–C). Signal intensities of hFc standard bands (one band at ~25 kDa each in Figure 4A, lanes 1–5) were linearly related to the quantity of hFc present (Figure 4C). Gal-1hFc (one band at ~40 kDa in Figure 4A, lane 6) and degraded Gal-1hFc (one band at ~40 kDa and one band at ~25 kDa in Figure 4A, lane 7) signals were within the standards range (Figure 4A, lanes 1–5). The concentration of purified Gal-1hFc (Figure 4A, lane 6) on the basis of hFc was determined to be 450 μg/ml by image processing (Figure 4C). Adjusting for the molecular weight of Gal-1hFc, the concentration of the purified material was 720 μg/ml. In contrast, too much purified Gal-1hFc was loaded in lanes 6 and 7 of the Western in Figure 4B, which generated signals that saturated or exceeded the hFc standards range, thereby preventing quantification. Alternative to Western blotting, Coomassie gel staining[21] can serve as a rapid quality control check and/or quantification method (Figure 4D). Note that a band ~10 kDa, in addition to bands and ~25 kDa and ~40 kDa, was present in the degraded Gal-1hFc sample (Figure 4D, lane 2). This band is degraded Gal-1hFc that was non-reactive with the detection antibody used in Western blots (Figure 4AB).

After quantification and final characterization of the purified Gal-1hFc, sterile BSA was added to achieve a final BSA concentration of 0.5%. The purified material was then aliquoted and stored at −20°C. In addition to Gal-1hFc, we have used this streamlined protocol to isolate double mutant Gal-1hFc and Gal-7hFc. It can also be extended to practically any molecule that possesses an Fc fragment (antibodies, Fc fragments themselves, other Fc fusion proteins, etc. of species and isotypes that have affinity for protein A[14–17]).

DISCUSSION

The protocol described herein was developed to rapidly isolate and characterize a chimeric fusion protein expressing human Fc (Gal-1hFc), without significantly compromising product recovery or inflating cost. The key components of the streamlined workflow are a protein A membrane adsorber for isolation, and semi-dry transfer and vacuum-assisted immunoblotting for characterization by Western blotting.

The dramatic decrease in processing time for isolation and characterization of Gal-1hFc from 300 ml of cell culture supernatant (approximately 9 hours in the streamlined protocol compared to 20+ hours in a traditional protocol) is largely attributable to incorporation of the aforementioned key components, particularly the membrane adsorber. At the maximum recommended flow rate of 10 ml supernatant/min, membrane adsorber loading can be completed in 30 minutes. Gal-1hFc recovery from protein A membrane adsorbers typically range from 10% to 30% in our labs, with ~30% recovery routinely achieved by experienced lab personnel when Gal-1hFc concentrations in cell culture supernatant are 5–10 μg/ml. At most, ~50% recovery has been achieved. Complete product recovery is virtually impossible due to inherent losses due to (a) specific retention of Fc-expressing protein by protein A on the membrane adsorber (Figure 3B and 3C) and (b) non-specific surface adsorption of the

protein throughout isolation. It is important for individual labs to determine if product recovery with a membrane adsorber is acceptable based on their needs; if altering loading flow rate or feedstock content (e.g., desired protein cell concentration culture supernatant, which may be particularly difficult) can improve product recovery[8], or if modification of the elution protocol (step 4) to obtain higher recovery is worth the increased time, expense, and effort, or the possible harm to the protein or membrane adsorber itself (primarily by elution buffer pH, salt concentration, etc.). In contrast to using the membrane adsorber, it takes approximately 10 hours to perform two perfusion cycles through a 1 ml protein A bead-packed column[18] at the maximum tolerable flow rate of 1 ml/min, and only 10–20% Gal-1hFc is typically recovered. As a final concern, a cost analysis for protein A beads from different manufacturers may affect the decision to use a standard bead-packed column versus a single membrane adsorber. Taking all of these factors into consideration, the use of a membrane adsorber for laboratory scale isolation of Fc-expressing proteins may offer small laboratories significant advantages over column chromatography[2,5,8,10].

Use of a mini gel for SDS-PAGE, semi-dry discontinuous electrophoretic transfer of proteins from the gel to PVDF membrane, and vacuum-assisted immunoblotting offer significant time savings compared to midi gels, tank (wet) transfer, and diffusion-based immunostaining for quantification and characterization of Gal-1hFc (2 hours versus 8 hours). Individual labs must determine whether the expedited Western protocol is appropriate for quantification and characterization of their protein. Benefits include (a) short time to completion even by inexperienced lab personnel, (b) ability to determine whether the protein is intact or has degraded due to processing or contamination (Figure 4B), and (c) relative ease of quantification by image processing programs (Figure 4C). However, (a) increased reagents and consumables costs may be unacceptable despite the time offset for wages, (b) traditional protein quantification by A280, ELISA, Bradford assay, or BCA assay may be preferred[23–27], or (c) labs may not have access to a semi-dry blotter or a vacuum-assisted protein detection system for immunoblotting. In such cases, Coomassie staining of SDS-PAGE resolved proteins[21] (Figure 4D) or dot or slot blotting[28] are good alternatives.

Flow cytometry was used in three separate 90-min tests for detection of functional Gal-1hFc in this streamlined protocol (Figure 2), which is the same as in traditional processing[18,22]. Any other characterization method such as Western blotting or ELISA would also be acceptable. Functional assays are optimal (e.g., detection of galectin-1 ligands on breast cancer cells, Figure 3A–C). Alternatively, detection of the Fc unit would be acceptable (similar to Figure 3D), but retention of the protein's specific function cannot be determined in this manner, which is a potentially significant drawback.

The methods described herein have been used for the rapid laboratory scale isolation and characterization of a chimeric fusion protein expressing human Fc (Gal-1hFc). This streamlined workflow can be applied to the isolation of practically any molecule that possesses an Fc fragment (IgG antibodies, other Fc fusion proteins, Fc fragments themselves, etc.) and can be readily modified based on individual lab preferences. In addition, this protocol is relatively fast and easy, even for limited-experience or completely inexperienced lab personnel, making it suitable for use in high school or college biology, chemistry, and (bio)chemical engineering courses that cover principles of protein isolation and characterization.

Acknowledgments

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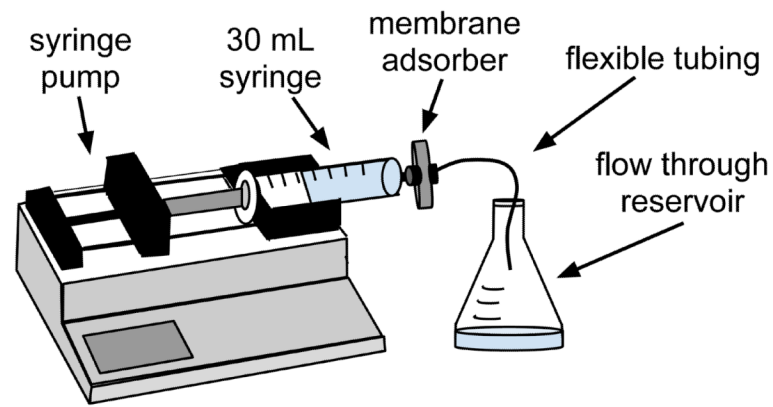
Figure 1. Schematic of the experimental apparatus
Antibodies and recombinant fusion proteins expressing Fc fragments of IgG can be isolated from dilute solutions using a protein A membrane adsorber. The membrane adsorber is connected through a Luer Lock fitting to a 30 ml syringe on a syringe pump, which perfuses the protein-containing solution through the membrane adsorber at a desired volumetric flow rate.
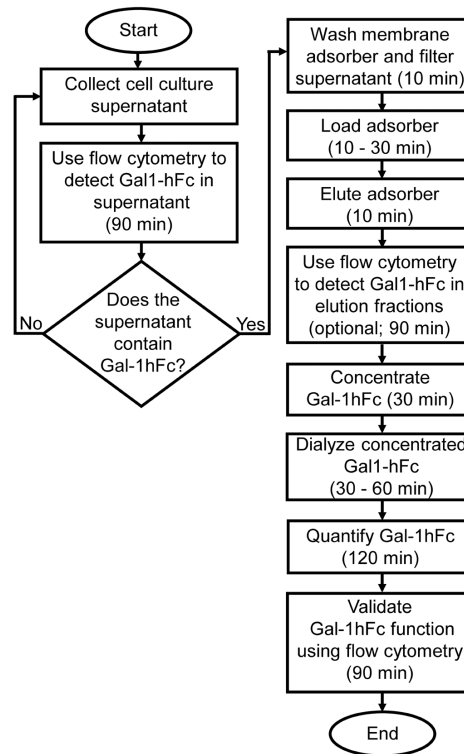
Figure 2. Process workflow for the isolation of Gal-1hFc from cell culture supernatant
This flowchart illustrates the protocol for the isolation and characterization of a chimeric murine galectin-1/human Fc-expressing fusion protein (Gal-1hFc) from cell culture supernatant. In general, processing time will vary depending on operator experience, amount of supernatant processed, and which quantification and characterization methods are used.
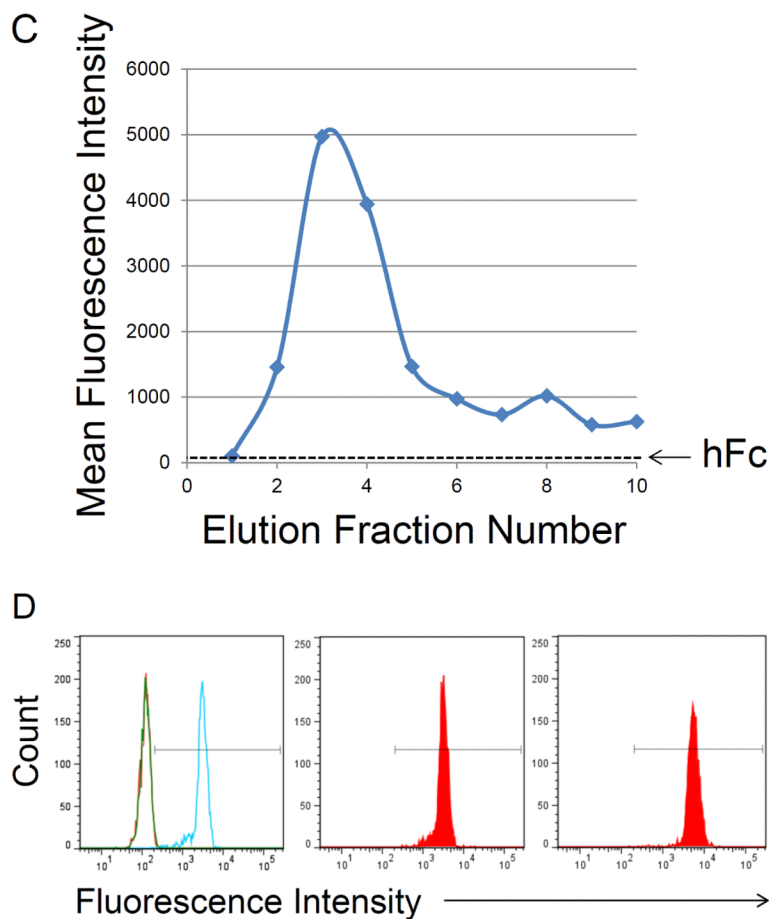
C

D
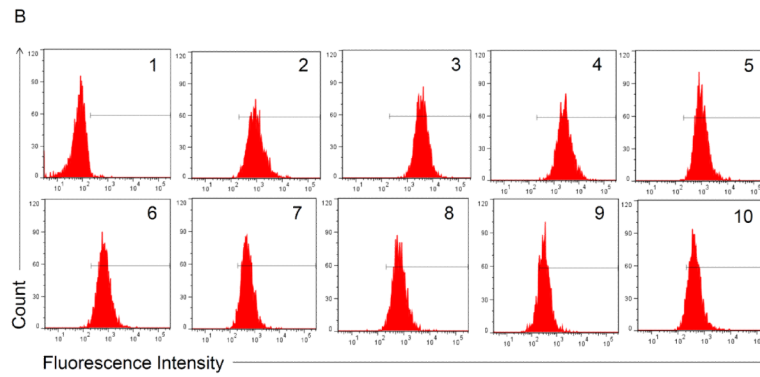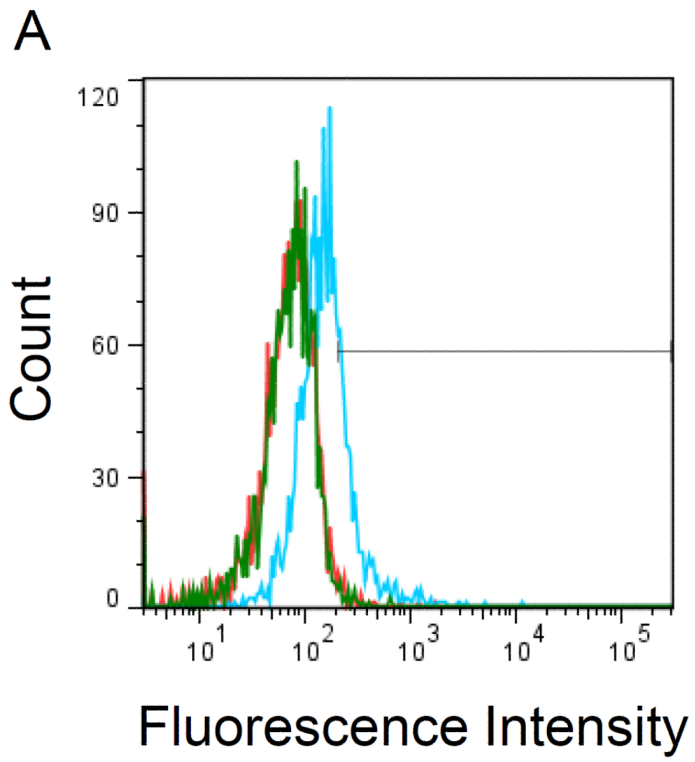
Figure 3. Flow cytometry analysis of Gal-1hFc-containing solutions
Flow cytometry is used to assess whether functional Gal-1hFc is present in fresh cell culture medium, cell culture supernatant, membrane adsorber flow through, and elution fractions. APC-conjugated F(ab')$_2$ anti-human Fc was used as the secondary antibody. All data were acquired using a FACSAria Special Order Research Product flow cytometer/sorter and analyzed using FlowJo software. Markers in each histogram represent 2% of negative control population (i.e., fresh cell culture medium for (A) and hFc isotype for (B)). (**A**) Flow cytometry histogram overlay of BT-20 breast cancer cells labeled with fresh cell culture medium (red), cell culture supernatant (blue), or membrane adsorber flow through (green). Cell culture supernatant contained a low level of functional Gal-1hFc that successfully bound to galectin-1 ligands on BT-20 cells, while the fresh cell culture medium and membrane adsorber flow through lacked Gal-1hFc, as expected. (**B**) Flow cytometry histograms of BT-20 cells labeled with elution fractions from the membrane adsorber. Gal-1hFc signal on the BT-20 cells sequentially increases to a maximum at elution 4 then decreases, although not to background level (i.e., signal equivalent to fresh cell culture medium). (**C**) The mean fluorescence intensities of the samples in (B) can also be plotted as a function of elution fraction number to generate an elution curve. The mean fluorescence intensity of cells labeled with hFc isotype control is shown as a reference line. (**D**) Alternatively, flow cytometry analysis of Gal-1hFc bound to protein A polystyrene beads can be used to test for protein presence, but not function, in various solutions. APC-conjugated F(ab')$_2$ anti-human Fc was used as the secondary antibody. Left: Flow cytometry histogram overlay of fresh cell culture medium (red), cell culture supernatant (blue), and blank buffer sample (green). Middle: Histogram of hFc isotype control. Incubation with 10

μg/ml hFc is a positive control for protein A capture. Right panel: Histogram of protein A beads incubated with purified Gal-1hFc, on the basis of 10 μg hFc/ml.
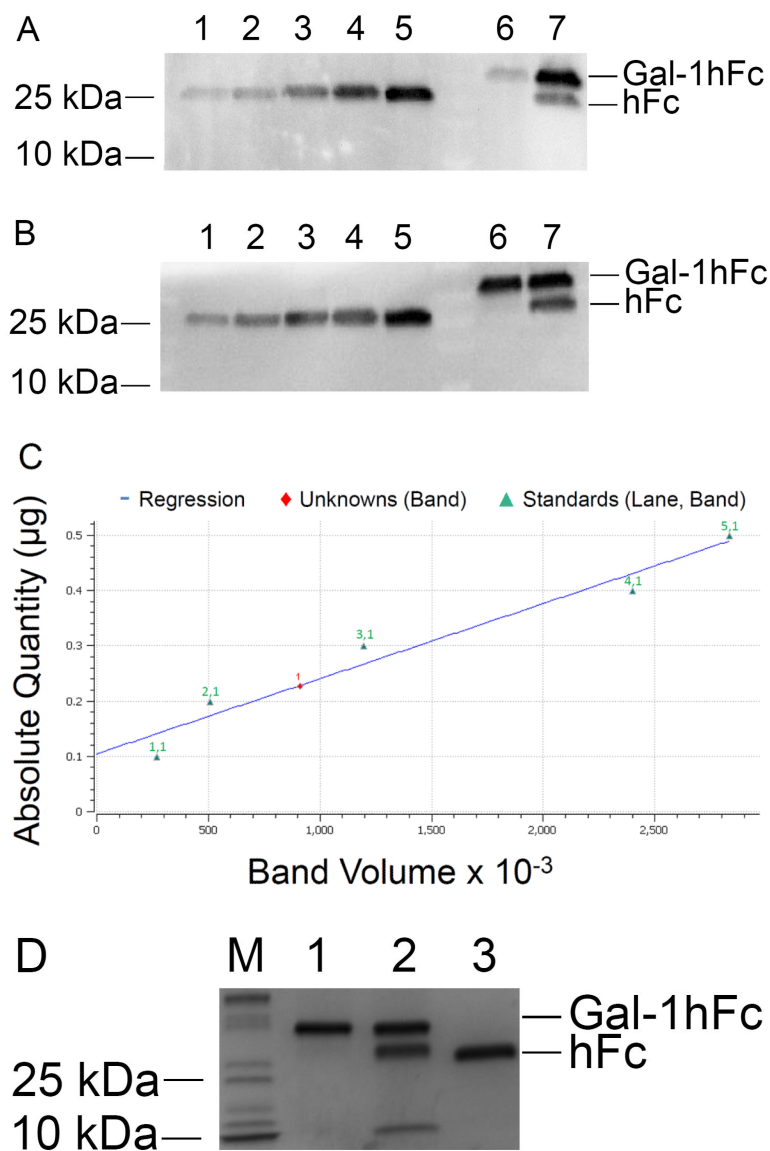
Figure 4. Quantification and characterization of Gal-1hFc using Western blotting and image analysis

(A) Lanes 1–5 are hFc standards of 0.1–0.5 µg in 0.1 µg increments, and lanes 6 and 7 are purified Gal-1hFc from two different production lots. Samples were resolved on a 4–15% TGX gel by SDS-PAGE under reducing conditions, then transferred to PVDF membrane using discontinuous transfer conditions. The membrane was blocked and incubated with anti-hIgG-AP using a vacuum-assisted immunoblotting technique. Signals were developed with ECL reagent and visualized on a Bio-Rad ChemiDoc XRS imager. Signals for hFc standards in lanes 1–5 varied linearly (plotted in (C)), and the signals for the two purified Gal-1hFc samples in lanes 6 and 7 were within the linear measurement range. Lane 7 shows two bands, Gal-1hFc and presumably hFc fragment, indicating degradation of the protein. **(B)** If too much purified Gal-1hFc is loaded, the signal may saturate or exceed the linear signal range, preventing quantification (lanes 6 and 7). Lanes, gel, and running conditions are the same as in (A). **(C)** Image Lab analysis software allows the input of known absolute quantities of hFc standards from an immunoblot, then determines the quantity of the

unknown band(s) through its signal relative to the linear regression of standards ($y = 1.36 \times 10^{-7} * x + 0.105$; $R^2 = 0.95$). Data shown are from the analysis of the immunoblot in (A). **(D)** Quality of purified Gal-1hFc can be assessed using Coomasie staining. Lane M is a molecular weight marker, lane 1 is purified Gal-1hFc (same sample as lane 6 in (A) and (B)), lane 2 is degraded Gal-1hFc (same sample as lane 7 in (A) and (B)), and lane 3 is 0.2 µg hFc standard. Gel and running conditions were the same as in (A) and (B).

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Protein A membrane adsorber (Sartobind Protein A 2 ml)	Sartorius- Stedim	93PRAP06HB-12--A	
Amicon Ultra centrifugal filter, 10 kDa, 4 ml	Millipore	UFC801008	Use 15 ml volume if needed
Sterile vacuum filter unit, 0.22 μm, 500 ml	Millipore	SCGPU05RE	
10 ml Syringes with Luer Lock tips	BD	301604	
30 ml Syringes with Luer Lock tips	BD	309650	
Tygon lab tubing (1/16"×1/8"×50')	Cole Parmer	WU-95903-16	
Slide-A-Lyzer MINI Dialysis Devices (10K MWCO)	Thermo Scientific	69576	
Snap i.d. antibody collection tray	EMD Millipore	WBAVDABTR	
Snap i.d. single well blot holder	EMD Millipore	WBAVDBH01	
Elution buffer	Thermo Scientific	21004	
Tris, ultra pure	Fisher Scientific	819623	
Sodium chloride	Fisher Scientific	7647-14-5	
Tween 20	Fisher Scientific	BP337-100	
DPBS	Thermo Scientific	SH30028.02	
DPBS with Ca^{2+}/Mg^{2+}	Life Technologies	14080-055	
BSA	Sigma	A9647	
Human Fc	Bethyl Research Laboratories	P80-104	
Anti-human Fc-APC	Jackson Immunoresearch	109136170	
Anti-human Fc-AP	Bio-Rad	170-5018	
Alkaline phosphatase substrate	Promega	S3841	
Flow cytometry tubes (5 ml polystyrene or polypropylene)	BD	352054	

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Syringe pump	Harvard Apparatus	55-2226	A peristaltic pump is also acceptable
Protein gel electrophoresis system	Bio-Rad Laboratories	552BR094876	Any gel electrophoresis system is acceptable, but mini gel systems run fastest
Semi-dry blotter	Bio-Rad Laboratories	690BR006163	Any transfer system is acceptable, but discontinuos transfer systems perform electrophoretic transfer fastest
SNAP i.d. vacuum-assisted protein detection system	EMD Millipore	WBAVDBASE	An upgraded model has replaced this specific instrument, but either should work just as well