Editorial Comments

Please add this to the reference list and use superscripted citation instead.

Reference has been added as follows:

FDA. *New Molecular Entity (NME) Drug and New Biologic Approvals*, <<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/NDAandBLAApprovalReports/ucm373420.htm>> (2015).

Should this step appear after 1.2.4?

The step has been moved as below:

1.2.5 Open the saved method file. Save the result file in the required location and specify file name when prompted. Hit run after the sample has been injected into the large volume sample loop.

Where is this from? Please add steps to describe how it is obtained and processed, and incubated etc. How are the cells cultured? Mention culture medium and conditions. What is the cell density?

We will reference the previous paper of which this paper is a continuation. The previous paper describes the cell culture and harvest process. A note has also been added with reference as below:

Protocol:

Note: The harvested cell culture fluid used in the following procedures has been obtained from chinese hamster ovary cells cultured in automated micro-bioreactors.

Replace with generic term

The following two terms have been replaced with generic terms as below:

Superloop: Large volume sample loop

LockSpray: Internal mass reference

What is the selected method? Unclear.

Response: The step has been clarified as below:

1.2.5 Open the saved method file. Save the result file in the required location and specify file name when prompted. Hit run after the sample has been injected into the large volume sample loop.

Please mention the software steps including button clicks and use the imperative voice.

Response: There is only one button click which is Run. The instrument runs the steps by itself. The step has been modified to indicate this.

1.3.1. Select the saved method and click run when prompted by instrument software(Refer 1.2.5).

Units? w/w? Mention molar concentration.

The units have been added as below:

Use a protein extinction coefficient of 1.37 mL\*(mg\*cm)-1 at 280 nm for a 1% (% m/v) IgG solution.

Examples? Which mAb? Please clarify. Also mention the concentration.

3.1.1 Start with antibody concentrations of 2 mg/mL in an appropriate buffer such as neutral sodium phosphate, citrate or HEPES buffer. Prepare an intact mAb standard (such as NIST mAb) at 2 mg/mL to process alongside the experimental samples to serve as a positive control.

Is the kit listed in the table of materials?

This is listed in the table of materials as GlycoWorks RapiFlour-MS N-Glycan Starter Kit.

Is this listed in the table of materials? If not, please add it and add the composition here.

Response: The fluorescent tagging reagent is included as part of the GlycoWorks RapiFlour-MS N-Glycan Starter Kit.

Section 3.2-If you wish to film the entire analysis the next few steps must be highlighted.

Section 3.2 has been highlighted again to include more steps.

Using which reagent?

3.2.1. Note: The column must be flushed with 60% acetonitrile and 40% H2O before use: 50 CVs before the first use or 20 CVs if the column has been used before.

We cannot film this unless details of the processing as described and highlighted. I have unhighlighted 3.3 and 3.3.1. Please do cite a reference for the processing.

Waters Corporation sells training for this data processing, but they don’t provide or sell training manuals for this.

what is the composition?

The labelling buffer and labelling reagent are included as part of the Protein Charge Variant Reagent Kit. The step has been modified as below:

5.1.3. Dilute the sample to a final concentration of 2 mg/mL in a volume of 25 μL and add 5 μL of the labeling buffer (see Table of Materials: Charge Variant Reagent Kit) in the 96-well plate. Prepare the labeling reagent by diluting the necessary amount of labeling reagent (see Table of Materials: Charge Variant Reagent Kit) 1:30 in dimethylformamide.

Step 6.2.3: when and where are the injected? And how much?

The volume of the injection is dependent on the calibration range of your standard curve and can range from 0.1 ul to 10 ul, as noted previously.