Binding Kinetics

Approvals: Release Date:
Preparer: Eben Crawford Expiration Date:
Reviewer:

1 Contents

2 Purpose

Identifying the intended use of the process.

To detail the procedure for performing a stirred cell binding kinetics experiment to quantify the rate of protein binding to chromatography resin.

3 Scope and Applicability

Describing the purpose of the process or procedure and any organization or regulatory requirements, as well as any limits to the use of the procedure.

This procedure applies to the Process Development Department members who are developing purification processes for CCTC operations.

4 Summary of Method

4.1 Conditions for the experiment are selected based on historical data or the project's *Work Breakdown Structure.* A combination of resin slurry and target protein solution is added to a mixing container and agitated for set time intervals. Following the set time intervals, samples are removed from the mixing container and filtered into sample tubes. The sample's protein concentration is then measured using the UPLC/HPLC ProA Column. From the protein data and time of sampling, the binding rate and capacity can be calculated.

5 Definitions

Defining any words, phrases, or acronyms having special meaning or application.

Word	Definition
Resin	Chromatography resin for biologic purification
Protein	The target protein in solution to be isolated by resin binding
Mixing Apparatus	A system for gently agitating resin slurry

Acronym	Definition
UPLC	Ultrahigh Performance Liquid Chromatography

6 Health and Safety Warnings

Indicating operations that could result in personal injury or loss of life and explaining what will happen if the procedure is not followed or is followed incorrectly; listed here and at the critical steps in the procedure.

All proper PPE procedures must be followed while working the wet lab.

When working with electrical systems it is always recommended to ensure that all wired connections are secure before connecting the system to power and that all power cables are properly sheathed and undamaged.

Ergonomic warning – sitting for more than a half hour can cause health hazards.

7 Cautions

Indicating activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at critical steps in the procedure.

Mixing equipment should be connected to a backup power supply such as APC Power Saving Back-UPS Pro 1500 (Item# BR1500G). Ensure that the mixing apparatus is stopped or that the user is able to keep hands away from moving parts when performing sampling.

8 Interferences

Describing any component of the process that may interfere with the accuracy of the final product.

Ensure that the time to draw a sample and filter it are accounted for in the targeting specific timepoints. For short sample intervals this can introduce error on the time scale.

When selecting a mixing apparatus ensure that mechanical mixing is free standing or from indirect contact. Mixing with two surface contacts will physically damage the resin causing the results to be void.

9 Personnel Qualifications/Responsibilities

Denoting the minimal experience the user should have to complete the task satisfactorily, and citing any applicable requirements, like certification or "inherently governmental function".

Operators should maintain a current training on lab safety and have familiarized themselves with this SOP.

Supervisors should make sure that operators maintain a current training and understand the SOP.

Managers should review the SOP on a regular basis, collect feedback from Operators and Supervisors, and either renew, revise, or phase out this SOP.

10 Equipment and Supplies

Listing and specifying, where necessary, equipment, materials, reagents, chemical standards, and biological specimens.

10.1 Equipment

Equipment Name	Product #	EQP ID #	Product #	EQP ID #
UV-Vis				
HPLC				
Hula Mixer				
Overhead Mixer				
Shaker Plate				
15 mL Conical Tube				
50 mL Conical Tube				
50 mL PETG Beaker				
100 mL PETG Beaker				
5 mL Syringe				
30 mL Syringe				
50 mL Syringe				
2 mL Sample tubes				
PES Syringe Filter				

11 Procedure

Identifying all pertinent steps in order and the materials needed to accomplish the procedure such as: Instrument or method calibration and Standardization, Sample Collection, Sample Handling and Preservation, Sample Preparation and Analysis (such as extraction, digestion, analysis, identification and counting procedures), Troubleshooting, Data Acquisition, Calculations & Data Reduction Requirements (such as listing any mathematical steps to be followed), Computer Hardware and Software (used to store field sampling records, manipulate analytical results and/or report data).

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11.1 Experiment Attachment Setup

11.1.1 Start the Binding Kinetics Attachment by filling out the first three fields in Table 1-1.

User	
Experiment Date	
Project	
Reviewed By	
Reviewed Date	

Table 1-1

11.1.1.1 User – The initials of the person performing the experiment

11.1.1.2 Experiment Date – The date that the experiment will be completed

11.1.1.3 Project – The name of the project that this experiment applies to

11.1.2 Fill out Tabel 1-2 with all available information for the experiment.

SOP	File
Feed Material	
Resin Prep	
Buffer Model	
Buffer Plan	
ProA	
Avery Labels	
Cryobaby Labels	

Table 1-2

11.1.2.1 Feed Material – Previous experiment or cell culture clarification data

11.1.2.2 Resin Prep – Resin Preparation Attachment for resin to be used in the experiment

11.1.2.3 Buffer Model – The corresponding buffer model for the CCTC operation the development is for

11.1.2.4 Buffer Plan – The range of buffers that are being considered for development

11.1.2.5 ProA- HPLC ProA Attachment for feed material and experiment samples

11.1.2.6 Labels used for experiment (Avery Template)

11.1.2.7 Labels used for experiment (Cryobaby Template)

11.1.3 In Table 1-3 provide links to all Buffer Preparation Attachments for buffers used in this experiment.

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SOP	File
Buffer Prep (1)	
Buffer Prep (2)	
Buffer Prep (3)	
Buffer Prep (4)	
Buffer Prep (5)	
Buffer Prep (6)	
Buffer Prep (7)	
Buffer Prep (8)	
Buffer Prep (9)	

Table 1-3

11.2 Experiment Plan

11.2.1 In Table 2-1, enter the conditions for the binding kinetics experiments to be performed.

	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)	Experiment (5)	Experiment (6)	Experiment (7)	Experiment (8)	Experiment (9)
Material Name									
Resin Name									
Resin Buffer									
Target Loading (g/L)									
Feed Conc (g/L)									
Starting Slurry Conc.									
Gamma	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
System Vol.									
Feed Volume (mL)	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Slurry Vol. (mL)	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**
Protein (mg)	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Exp Resin Concentration	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Table 2-1

11.3 Feed Material Preparation

11.3.1 Ensure that the protein feed solution has been filtered using a syringe or bottle top PES filter with a pore size of 0.22 um.

11.3.2 Transfer the target feed volume for the experiment into a clean conical tube or beaker.

11.3.3 Record the *Gross* and *Tare* weights in Table 3-1.

Start Feed Material	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)	Experiment (5)	Experiment (6)	Experiment (7)	Experiment (8)	Experiment (9)
Gross									
Tare									
Net	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g

Table 3-1

11.3.4 If no adjustments will be made to the protein feed solution, continue to section 11.2.12.

11.3.5 If the protein solution will be adjusted for pH or conductivity enter the target pH or conductivity for the adjusted protein solution.

Feed Adjustment	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)	Experiment (5)	Experiment (6)	Experiment (7)	Experiment (8)	Experiment (9)
Target pH/Cond									
Starting pH/Cond									
Adjusted pH/Cond									
Buffer									
Gross									
Tare	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
Net	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
Dilution	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
New Gamma	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Table 3-2

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11.3.6 Record the *Buffer* to be used for protein feed adjustment into Table 3-2.

Feed Adjustment	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)	Experiment (5)	Experiment (6)	Experiment (7)	Experiment (8)	Experiment (9)
Target pH/Cond									
Starting pH/Cond									
Adjusted pH/Cond									
Buffer									
Gross									
Tare	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
Net	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
Dilution	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
New Gamma	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Table 3-2

11.3.7 Measure the initial value of the protein solution following (SOP-EQ-MS-001) for pH or (SOP-EQ-MS-002) for conductivity.

11.3.8 Titrate protein solution until the target value is achieved.

11.3.9 Record the adjusted pH or conductivity value of the protein solution in *Adjusted pH/Cond* Table 3-2.

11.3.10 Record the *Gross* weight of the adjusted protein solution in Table 3-2.

11.3.11 If the final vessel for the experiment will be different than the measuring vessel or a dilution was performed, use an analytical balance to record the *Gross* and *Tare* values of protein solution that will be used for the binding kinetics experiment.

11.3.11.1 If no adjustment is made the *Net* weight of the protein solution from Table 3-1 will populate in *Net* of Table 3-3.

11.3.11.2 The *Target Protein Volume (mL)* will display the expected protein solution needed for the experiment.

Final Feed	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)	Experiment (5)	Experiment (6)	Experiment (7)	Experiment (8)	Experiment (9)
Gross									
Tare									
Net	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
Target Protein Volume (mL)	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Concentration	0.000 g/L	0.000 g/L	0.000 g/L	0.000 g/L	0.000 g/L	0.000 g/L	0.000 g/L	0.000 g/L	0.000 g/L
Product	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg

Table 3-3

11.3.12 Gently mix the stock of resin prepared at the target slurry (%) following SOP-LG-RP-001.

11.3.13 When the resin slurry has been thoroughly mixed so that resin is uniformly suspended, transfer the *Target Resin (mL)* volume into a measuring vessel.

11.3.14 Record the *Gross* and *Tare* weight of resin removed from the stock container in Table 3-4.

Resin	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)	Experiment (5)	Experiment (6)	Experiment (7)	Experiment (8)	Experiment (9)
Gross									
Tare									
Net	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g
Target Resin (mL)	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Slurry Conc.	0%	0%	0%	0%	0%	0%	0%	0%	0%
Resin Volume	0.00 mL	0.00 mL	0.00 mL	0.00 mL	0.00 mL	0.00 mL	0.00 mL	0.00 mL	0.00 mL
Loading	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
System Volume (mL)	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g

Table 3-4

11.4 Experiment Execution

11.4.1 Each experiment has a data entry section starting in section 5.

11.4.2 Set the sample schedule in the *Sample Time* column of Table (5-13)-3.

11.4.2.1 Example sample times are provided for each experiment.

Experiment (1)							
Sample Time	Reading (1)	Reading (2)	Sample Dilution	Avg. Protein Conc. (g/L)	Recovery %	Bound Protein (g/L)	Binding Rate (mg/mL/min)
0.0 min				#DIV/0!	0.00%	0.00	0.00
0.5 min							
1.0 min							
1.5 min							
2.0 min							
2.5 min							
3.0 min							
5.0 min							
10.0 min							
15.0 min							
20.0 min							

Table 5-3

11.4.3 Mix the resin slurry and the protein solution together in the experiment vessel and set it to mix on one of the mixing systems from Section 10.1.

11.4.4 Using a clean 5mL syringe draw 2mL samples from the experiment vessel at the planned intervals for the experiment in Table (5-13)-3.

11.4.5 Filter the sample through a clean 0.22 um PES syringe filter into 2mL cyrovials labeled with:

- Project
- Resin
- Experiment ID
- Date

11.5 Experiment Sample Processing

11.5.1 Select the *Protein Detection Method* in Table (5-13)-1 for the binding samples.

Protein Detection Method	Resin Buffer	Resin Type	Resin Loading
HPLC	0	0	#DIV/0!

Table 5-1

11.5.1.1 If UV-Vis is used enter the *Blank*, *Extinction Coefficient*, and *Pathlength (cm)* for the UV detector in Table 4-1.

Blank	Extinction Coefficient	Pathlength (cm)

Table 4-1

11.5.2 Measure the protein concentration using the method selected in Table (5-13)-1.

11.5.2.1 Follow SOP-PD-QC-002 ProA U-HPLC or SOP-PD-QC-003 SEC U-HPLC when selecting HPLC for the *Protein Protection Method.*

11.5.2.2 Follow SOP-PD-QC-001 UV-Vis when selecting UV-Vis for the *Protein Protection Method.*

11.5.3 Enter the measured concentration next to their corresponding sample times in Table (5-13)-1.

11.5.3.1 If UV-Vis is used for detection enter the *Dilution* factor used for each sample in Table (5-13)-1.

11.6 Experiment Cleanup

11.6.1 Using 1x PBS and a 30 mL syringe, backflush the filters used for sampling with 5 mL of buffer to collect trapped resin.

11.6.2 Combine the remaining volume from the experiment vessel and the resin collected from the filters into one PETG container with a lid.

11.6.3 Clean and exchange resin into storage buffer following SOP-LG-RP-001.

11.6.4 Put plasticware into washroom.

11.6.5 Dispose of filters and syringes used in the experiment.

12 Data and Records Management

Identifying and calculations to be performed, forms to be used, reports to be written, and data and record storage information.

12.1 Data Retention

12.1.1 Naming Convention

- Project ID
- Experiment Name
- Experiment Serial Number
- Date

Example: ChromaTan-01 Binding Kinetics-01 08-Aug-21

12.2 Process Data

12.2.1 Each experiment has 3 plots for Protein Concentration, Resin Bound Protein, Protein Recovery % vs Sample Time.

12.2.2 In the Aggregated Data Plots tab, the 3 standard plots are overlaid with every experiment in the workbook.

13 QA/QC Considerations

QC activities are designed to allow self-verification of the quality and consistency of the work. Describe preparation of appropriate QC procedures (self checks, such as calibration, recounting, reidentification) and QC material (such as blanks – rinsate, trip, field or method; replicates; splits; spikes; and performance evaluations samples) that are required to demonstrate successful performance of the method. Describe the frequency of required calibration and QC checks and discuss the rationale for

decisions. Describe the limits/criteria for QC data/results and actions required with QC data exceed QC limits or appear in the warning zone. Describe the procedure for reporting QC data and results.

13.1 If an error occurs prepare an error report detailing the nature of the error, potential cause(s) of the error, and potential corrective action(s).

14 References

Documents or procedures that interface with the SOP should be fully referenced (including version), such as related SOPs, published literature, or method manuals. Citations cannot substitute for the description of the method being followed in the organization. Attach any that are not readily available.

14.1 Benchtop pH Measurement

14.1.1 SOP-EQ-MS-001

14.2 Benchtop Conductivity Measurement

14.2.1 SOP-EQ-MS-00

14.3 Resin Exchange and Preparation

14.3.1 SOP-LG-RP-001

14.4 UV-Vis Protein Reading

14.4.1 SOP-PD-QC-001

14.5 ProA U-HPLC

14.5.1 SOP-PD-QC-002

14.6 SEC U-HPLC

14.6.1 SOP-PD-QC-003