# Protein purification lab report

*Activities 4.1.3 and 4.1.4. Did the product pass, and how do you know?*

This is the report a manufacturing laboratory actually writes. Not 'here is what I did' but 'here is the evidence that what came out of the column is pure enough to put in a person'. Purity is not a feeling. It is a band count on a gel.

HELPS ON THE WEBXAM. A LOT | 90 MINUTES TO TWO HOURS | OFTEN TWO SITTINGS | NEEDS YOUR FRACTION SHEET AND YOUR GEL

## What this is

A full lab report on the purification you ran: how you did it, which fractions held your protein, how pure the gel says they are, and a quality control verdict that cites the gel rather than asserting a feeling.

This write-up used to sit on 4 December. It came off the calendar so that **how a column separates one protein** could take a full block on 2 December, the class day before you run the column, instead of being only an evening's reading. Running a chromatography column while still working out what the resin is doing is how fractions get collected in the wrong order.

**DOES THIS HELP ON THE WEBXAM? YES, ON THE TWO DOMAINS THAT TOGETHER ARE MOST OF THE EXAM**

**Bio-Molecular Technology is 57.73 percent of WebXam 072130**, the largest thing on it. Column chromatography, fraction collection and gel electrophoresis of proteins all sit inside that domain, and this report makes you use all three in one argument.

The second half lands in **Laboratory Standard Operational Procedures, 14.43 percent**. Purity standards, what counts as acceptable, what a contaminant would do to a patient, and how you controlled for error are all SOP questions. Writing a quality control verdict is practising the exact reasoning that domain tests.

## What you need in front of you

**GATHER THESE FIRST**

- **Your fraction collection data sheet:** tube number, buffer applied, whether the tube glowed under UV, and which tubes you labelled as target.
- **Your error control note** from the lab day.
- **Your annotated gel image** with the marker lane labelled, your size estimate, and the band count for each fraction.
- **Your purity notes** on what else is in a cell mixture besides the protein you want.

**Missing the gel?** Ask Mr. Mendoza for the class gel image. Working from a shared image is fine as long as your report says which image it is.

# What a purity argument is made of

**Fraction**

One numbered tube of liquid coming off the column. Fractions are collected in order, so the tube number is a record of time as well as volume.

**Elution**

Releasing the bound protein from the resin by changing the buffer. What comes off during elution is what you were trying to keep.

**Marker lane**

The lane of known protein sizes on the gel. Every size estimate you make is a comparison against it, so a report that does not mention it has no scale.

**Band count**

How many distinct bands a lane shows. One band is one protein species. Extra bands are extra proteins, which is what impurity looks like.

**Quality control verdict**

A stated pass or fail against a stated standard, with the evidence attached. A verdict without a standard is an opinion.

### WHAT A QUALITY CONTROL VERDICT SOUNDS LIKE

Set your standard first. A standard invented after you look at the gel is not a standard.

> **Not a verdict.** 'The purification worked well and the protein was pure.'
>
> **A verdict.** 'I set the standard before looking: a lane passes if it shows one clearly dominant band at the expected size and no more than one faint additional band. One of my eluted lanes meets that. The lane immediately before it does not, because it shows three bands of comparable intensity, so I am reading it as the tail of the wash rather than clean product. On that standard the purification passes, and the only material I would carry forward is the single lane that met the standard.'
>
> Notice what makes the second one a verdict. The standard is stated before the result, the evidence is a band count, and a lane is rejected for a stated reason. Your own report names the actual tube numbers. This example does not, because your tube numbers depend on your run and nobody else's.

## Build it in order

1. **Write the methods summary in order, in the past tense.** Column set up, mixture loaded, wash buffer applied, elution buffer applied, fractions collected and numbered, fractions checked under UV. Short sentences. The test is whether somebody could repeat it from your paragraph alone.

   **Done when:** your methods paragraph contains every step in the order it happened

2. **Rebuild your fraction table.** One row per tube: tube number, which buffer was on the column at the time, whether it glowed, and whether you called it target. Include the tubes that did nothing.

   **Done when:** every tube you collected has a row

3. **Write your purity standard down before you look at the gel again.** One sentence. How many bands, at what relative intensity, would you accept? Write it, then go and look.

   **Done when:** your standard is written and dated before your result paragraph exists

4. **Read the gel against the marker lane.** Estimate your protein's size, then count bands in each fraction lane. Write the counts into your table as a new column.

   **Done when:** you have a size estimate with the marker lane named as the reference

5. **Write the results paragraph.** Which fractions held the protein, what the UV signal said, what the gel said, and where those two sources of evidence agree or disagree. If they disagree, that is the most interesting sentence in your report. Do not smooth it over.

   **Done when:** both the UV evidence and the gel evidence appear, and any disagreement is named

6. **Write the quality control verdict.** Pass or fail against the standard you wrote in step 3, citing the band count. Name the fraction you would carry forward and the fractions you would discard.

   **Done when:** your verdict names your standard, your evidence, and a specific fraction

7. **Write one paragraph on why purity matters here.** Not in general. For a protein that is going to be injected into a patient, say what a contaminant left in the vial could actually do.

   **Done when:** you name a concrete consequence, not 'it would be bad'

8. **Write one error source and how you controlled for it.** Real candidates: fractions collected in the wrong order, the column running dry, a tube mislabelled, UV judged by eye in a bright room.

   **Done when:** your error source is specific to this procedure

9. **Name, period, attach the annotated gel, submit.**

   **Done when:** the marker lane is labelled on the image you attached

## Fill this in as you go

YOUR FRACTIONS, WITH THE GEL EVIDENCE ADDED

| TUBE | BUFFER ON THE COLUMN | GLOWED UNDER UV | BANDS IN THIS LANE | TARGET, WASH OR DISCARD |
|---|---|---|---|---|
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YOUR PURITY STANDARD, WRITTEN BEFORE YOU LOOKED

YOUR QUALITY CONTROL VERDICT

## If you get stuck

**My UV result and my gel disagree.**

Excellent. Write that up. A fraction can glow because the tagged protein is present and still show several bands because other proteins came off with it. Glowing tells you your protein is there. The gel tells you what else is there. They answer different questions and saying so is the sharpest paragraph in the report.

**None of my fractions glowed.**

Report it and troubleshoot on paper. Possible causes worth writing down: the protein never bound, the elution buffer never reached the resin, the fractions were collected before elution started, or the UV lamp was being used in room light. Pick the one your own notes support and say why.

**My gel has smears instead of bands.**

Describe what you see rather than pretending it resolved. A smear usually means overloading or degradation. Say which your data supports and what you would change on a second run.

**I cannot estimate the size.**

Line your band up against the marker lane and find the two marker bands it sits between. Report a range. A range with the marker named beats a single number with no reference.

**Is it a fail if my protein was not pure?**

No. It is a fail only against a standard you set, and reporting a fail correctly is worth full credit. What loses credit is asserting a pass with no standard and no band count.

## What to hand in

THE FINISHED LAB REPORT

- **Where it goes.** The same place as everything else. Your class form on the portal, or handed to Mr. Mendoza in person. Nothing about extra credit needs a different route.
- **What to hand in.** Your written pages, plus photographs of anything the handout asks you to photograph. Photographs of handwritten pages are fine. A photograph you cannot read is not.
- **Put your name and period on every page and every photograph filename.** Unlabelled work cannot be credited and it is the single most common way points get lost.
- **When.** Any day after the pack opens, right up to the last week of the semester. There is no deadline inside that window and no penalty for going slowly.
- **Feedback.** You get it back with a note. If a piece is short of full credit you may fix the named thing and resubmit once.

HOW BIG THIS ITEM IS: THE LARGEST ITEM IN THIS PACK

About 90 minutes to two hours, usually across two sittings. It is longer than the others because it carries two separate arguments, one about where the protein went and one about how pure it is, and both need evidence attached. No laboratory time is required.

**The point value is printed on the portal the day this item opens**, on the item's own card and in the gradebook entry. It is not printed here, because a number on paper and a number in the gradebook that disagree is worse than no number at all. Extra credit in this class is capped per semester, so check the cap on the portal before you start a fourth item.