

Required response record

MI / Genetics of Disease - September 23, 2026 - Route P: paper route

Your Wet ELISA data table record

the same six-task record, filled from the supplied Strip 1

One record with six tasks, T1 to T6: two control predictions, a six-well data table, the step order with one wash sentence, a control check, a flagged-strip check and one limit sentence

You read supplied illustrative records. Nothing in your record claims you ran a kit, observed a plate or measured anything. The color words are supplied observations. No sample well is called positive or negative today.

Today's six wells carry no concentration label and no unit. A clean control pair lets you read the other wells. It does not make any sample well positive or negative, and it does not guarantee that every well behaved. Calling a sample well positive or negative needs the test's decision rule, the stated cutoff for what counts, and that rule is not supplied today. A classroom kit is also not a test of a person: in a clinic a screening ELISA is not a diagnosis by itself, and a confirmatory test follows it.

Use your period and code, not your name.

Period and code

Date

Tasks included: T1 T2 T3 T4 T5 T6

Route: paper handed in, or one PDF

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R1. Your one record

Complete one Wet ELISA data table record with six tasks, T1 to T6: your two control predictions, a six-well data table, the step order with one wash sentence, a control check, a check of a flagged strip, and one limit sentence. Keep the six tasks together; they are one submission, on paper or as one PDF.

Your route line tells you where your well colors come from. On Route W you run a classroom kit with your group and read your own wells. On Route P you read a supplied illustrative record called Strip 1. The six tasks, the table and the limit are the same on both routes. Nothing on Route P claims you ran a kit, and nothing on either route calls a sample well positive or negative.

Thursday's paper dilution plan and Friday's four labeled model wells were separate records. Today's six wells carry no concentration label and no unit. You will record color words, compare each well with the negative control, and check the two controls. Tuesday's debate weighed the harm of false results. Today you see what a run has to show before anyone can trust a color at all.

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R2. The binding chain

An antigen is a substance an antibody can bind. An antibody has a binding site that contacts a particular part of the antigen. Binding is selective, but an antibody can sometimes bind another target; that is cross-reaction, and it is one reason a specific test is still not an error-free test. Janeway, Immunologists' Toolbox, introductory Immunization paragraphs and A-8.

In a color-based ELISA the layers stack in a set order. Antigen, or a sample that may hold it, goes into the well and attaches to the surface. A primary antibody is added and binds antigen if antigen is there. An enzyme-linked antibody is added and binds the antibody layer below it. Last, a substrate is added; the enzyme turns it into a colored product, so color appears where the whole chain is present. Color is the signal, the detectable response of the test. ELISA background, Specimen Requirements and Procedure; Assay Guidance Manual, Sandwich Immunoassay (ELISA).

Kits differ. Some attach an antibody to the well first and add the sample second; some name the steps differently. On Route W, your kit's printed procedure is the order you follow and record. On Route P, the supplied step order in R3 is the one you record.

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R3. Order and washing

Each reagent can only bind the layer added before it. Add a reagent out of order and it has nothing to hold onto; it washes away, or it stays behind as loose material that has nothing to do with binding. Either way the final color stops meaning what it should.

Between the main steps the wells are washed: liquid is added and emptied to carry away everything that did not bind. That is what makes the color specific. Skip a wash, or shorten it, and enzyme-linked antibody that never bound anything can stay in the well and make color where there is no antigen. ELISA background, Specimen Requirements and Procedure (between each of the four primary steps, a wash step removes unbound material); Assay Guidance Manual, Basic Steps for Developing and Running an Immunoassay.

The classroom model order, used for T3 on Route P and for comparison on Route W:

Incubation means the waiting time that lets a layer bind before the next step. On Route W, use the times printed in your kit's procedure and record when each one started. This reading does not give you a time; the kit does.

The classroom model order, used for T3 on Route P and for comparison on Route W:

Step 1. Add Antigen or sample. It binds the well surface. Then: Wash.

Step 2. Add Primary antibody. It binds antigen, if present. Then: Wash.

Step 3. Add Enzyme-linked antibody. It binds the antibody layer below. Then: Wash.

Step 4. Add Substrate. It binds the enzyme turns it into color. Then: Read; stop step only if a kit prints one.

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R4. Two controls decide whether to read the rest

A control is a well with an expected result, run the same way as every other well. The positive control holds material that should complete the chain, so it should develop color. The negative control holds no antigen, so it should stay nearly clear. Both are recommended for tests that give a yes-or-no style result, and both are treated exactly like the samples. ELISA background, Quality Control and Lab Safety; Assay Guidance Manual, Control Samples.

Read the two controls before you read anything else. If the positive control developed color and the negative control stayed nearly clear, the run passed its own check and you can read the sample wells. If the negative control developed color, the run made color without antigen; contamination, a skipped wash, a reused tip or a timing slip can all do that. The sample wells on that strip cannot be read, because the same problem could be adding color everywhere. The run is flagged, and a careful group repeats it or asks the teacher to review it. Do not subtract the negative control's color and keep going.

A clean control pair lets you read the other wells. It does not make any sample well positive or negative, and it does not guarantee that every well behaved.

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R5. Read color, then stop

Use four words for every well: Nearly clear, Light, Medium, Dark. They are an observation scale, in order, not amounts. Write the hue (for example, blue) once on the hue line above your table, because kits differ. Read each well on its own against a white background, then write whether it shows more color than the negative control, about the same, or less. Light is still more color than Nearly clear.

Today's wells have no concentration label. The darker-means-more relationship you used on Friday was that model's stated relationship, not a rule for every format, and some formats run the other way. Assay Guidance Manual, Sandwich Immunoassay and Competitive Binding Assay. You are not estimating an amount, and there is no curve to read one from.

"More color than the negative control" is a comparison you wrote down. It is not a positive result. Calling a sample well positive or negative needs the test's decision rule, the stated cutoff for what counts, and that rule is not supplied today. A classroom kit is also not a test of a person: in a clinic a screening ELISA is not a diagnosis by itself, and a confirmatory test follows it. ELISA background, Clinical Significance. Your T6 sentence names one thing the record cannot establish. Naming it is enough; do not invent the missing rule.

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R6. Your route

Only your route's section is on your page. If you were absent, or the class ran Route W and you did not, use R6-P.

R6-P. Route P: reading a supplied record

Strip 1 is a supplied illustrative record: six wells, a hue, and a color word for each well. It is what a careful group might have written down; it is not a plate you looked at and not a measurement. You copy its colors into T2, compare each with NC, list the supplied step order in T3, and check its controls in T4 exactly as a bench student would. Strip 2, the flagged strip in T5, is supplied to everyone on both routes.

Nothing on this route claims you ran a kit, opened a reagent, or observed a plate. Write "Supplied Strip 1" on your record's route line. If a well word seems odd, record it as supplied and use your T6 limit; do not change a supplied word.

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F1. Build, wash, read, check

Workflow for the six-well record. This is a teaching diagram, not a kit procedure, a reagent list or a picture of a plate. On Route W your kit's printed procedure supplies the actual steps and times; on Route P the supplied step order in R3 is what you record.

BUILD THE CHAIN, ONE LAYER AT A TIME

- Step 1 antigen or sample goes into the well
wash: unbound material leaves
- Step 2 primary antibody binds antigen, if antigen is there
wash
- Step 3 enzyme-linked antibody binds the layer below it
wash
- Step 4 substrate: color is meant to appear where the chain is complete, unless the run has an error
(stop step only if the kit prints one)

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READ EACH WELL ON ITS OWN

Nearly clear / Light / Medium / Dark
then compare with NC: more color / about the same / less

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CHECK THE TWO CONTROLS FIRST

PC developed color? NC stayed nearly clear?
Both yes: read the sample wells.
NC colored: the strip is flagged; repeat or review, do not subtract.

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STATE THE LIMIT

More color than NC is a comparison you wrote down.
Positive or negative needs a decision rule, which is not supplied.
A classroom kit is not a test of a person.

Full text equivalent. The chain is built one layer at a time, and between layers the wells are washed so that anything that did not bind is carried away. Antigen or sample attaches to the well. Primary antibody binds antigen if it is there. Enzyme-linked antibody binds the antibody layer below it. Substrate is added last, and the enzyme is meant to make color where the full chain is present, unless the run has an error; some kits print a stop step after that. Read every well on its own, using the four color words, then compare each well with the negative control. Before reading any sample well, check the two controls: the positive control should have developed color and the negative control should have stayed nearly clear. If both did, the sample wells can be read. If the negative control developed color, the strip is flagged, and the group repeats the run or asks for review rather than subtracting the color. Whatever the colors show, the record stops at a comparison. A positive or negative call for a sample well needs the test's decision rule, which this record does not supply, and a classroom kit does not test a person.

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T1. Predict the two controls

T1. Predict the two controls. Before any color is read, write what the positive control well should look like at the end of the run and what the negative control well should look like. Then write one sentence saying why a run needs both.

Space: 3 writing lines. Expected is not observed. Do not fill this from the data table.

Where it comes from: R2 and R4.

Positive control expected:

Negative control expected:

Why a run needs both:

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T2. Six-well data table

Route line: Route P: supplied Strip 1

Record source: Supplied Strip 1 (illustrative)

Hue supplied: blue

One line above the table: the color's hue, written once (Route W as observed; Route P supplied as blue).

Color words, in order: Nearly clear, Light, Medium, Dark. Compared with NC: More color than NC, About the same as NC, Less color than NC.

Fill one row for each of the six wells: the color you record using the four color words, and whether the well shows more color than the negative control, about the same, or less. Write the hue once on the hue line. Do not assign a concentration or a positive or negative call to any well.

Space: Six printed rows with blank Color and Compared-with-NC cells at least 3 cm wide and an optional Note column; one route line and one hue line above the table, and on Route W two more lines above the table for the kit name and the incubation start times. Read each well on its own before comparing. Light is still more color than Nearly clear. The four words are an observation scale, not amounts.

Where it comes from: Strip 1, the supplied illustrative record printed in the table area; you are copying supplied observations, not observing a plate.

Supplied Strip 1 (illustrative). Hue: blue.

Supplied illustrative observation words, not measurements; no concentration, no decision rule, no positive or negative call for any sample well; nothing claims a student ran a kit

Well	Contents as labeled	Color (supplied)	Hue
PC	Positive control	Dark	blue
NC	Negative control	Nearly clear	blue
S1	Sample 1	Dark	blue
S2	Sample 2	Nearly clear	blue
S3	Sample 3	Light	blue
S4	Sample 4	Medium	blue

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The six-well table

Well ID	Contents as labeled	Color	Compared with NC	Note
PC	Positive control			
NC	Negative control			
S1	Sample 1			
S2	Sample 2			
S3	Sample 3			
S4	Sample 4			

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T3. Order and wash

T3. Order and wash. List the main steps of the run in the order they happened, with a wash between each (four in the model; use your kit's count if it prints more). Then write one sentence: what does the wash remove, and what would a skipped wash do to the color?

Space: 4 short lines plus 2 writing lines. Your kit may name a step differently. Keep the order it printed. The wash sentence is about what the color would mean, not about being neat.

Where it comes from: R3's supplied classroom model order.

Template: The wash removes _____, so a skipped wash would _____.

Steps in order:

Wash sentence:

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T4. Control check

T4. Control check. Answer two questions from your table: Did the positive control develop color? Did the negative control stay nearly clear? Then write one sentence saying whether the other four wells can be read from this run, and why.

Space: 2 short lines plus 2 writing lines. A clean pair lets you read the other wells. It does not make them positive or negative.

Where it comes from: Strip 1's PC and NC rows as you copied them into T2.

Template: The positive control _____ and the negative control _____, so the other wells _____ because _____.

PC developed color: yes or no:

NC stayed nearly clear: yes or no:

Can the sample wells be read, and why:

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T5. Flagged strip, Strip 2

Supplied Strip 2 (illustrative, flagged). Hue: blue.

Supplied illustrative observation words; the negative control developed color, so the strip is flagged; no positive or negative call for any sample well

Well	Contents as labeled	Color (supplied)	Hue
PC	Positive control	Dark	blue
NC	Negative control	Medium	blue
S1	Sample 1	Dark	blue
S2	Sample 2	Medium	blue
S3	Sample 3	Light	blue
S4	Sample 4	Medium	blue

The run is flagged, and a careful group repeats it or asks the teacher to review it. Do not subtract the negative control's color and keep going.

T5. Flagged strip, Strip 2. Strip 2 is a supplied illustrative record for every student. Its negative control is Medium. In two sentences, say what that means for reading the sample wells S1 to S4 on that strip, and what should happen next with the run.

Space: 4 writing lines. Do not subtract the negative control's color and keep going. Say what the run shows and what a careful group does with it.

Where it comes from: Strip 2, supplied to everyone.

Template: Because Strip 2's negative control is Medium, _____. Next, _____.

What the Medium negative control means for the sample wells:

What happens next:

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T6. One limit

T6. One limit. Write one sentence naming something this record cannot establish about a sample well, or one kind of information that would be needed to establish it. Do not invent that information.

Space: 2 writing lines. More color than the negative control is a comparison you wrote down. A positive or negative call needs the test's decision rule, and a classroom kit is not a test of a person.

Where it comes from: Your own record and R5.

Template: This record cannot establish _____ because it does not supply _____.

Limit sentence:

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Before you submit

Read the record once as a stranger would

- ☐ I predict what both controls should show before I read any color.
- ☐ I record each of six wells with the four color words and compare each with the negative control.
- ☐ I list the steps in the order they ran and explain what the wash removes.
- ☐ I read the two controls first and say whether the other wells can be read.
- ☐ I explain what a colored negative control does to a strip.
- ☐ I name one thing my record cannot establish, without inventing the missing rule.

Complete one Wet ELISA data table record, T1 to T6. Hand in paper before leaving or submit one PDF to the assignment. Keep the record; Friday's specificity and sensitivity lesson works with ELISA records, and no sample well is called positive or negative until a decision rule is supplied.

Hand the paper record to Mr. Mendoza before leaving, or submit one PDF to Wk5 Data table: Wet ELISA lab by the due time on the assignment. Use your period and code. Do not submit twice.

Wk5 Data table: Wet ELISA lab

<https://clevelandmetro.schoology.com/assignment/8520303213>

Today's portal lesson

<https://mendozabiomed.org/learn/pltw-gend/day/2026-09-23>

Guided notes and a partner check are optional and are not another submission.