



User Manual Supplement for β -galactosidase Detection in Yeast Extracts

(PT3037-1)

**Catalog #: K2048-1 Luminescent
 β -galactosidase
Detection Kit II**

FOR RESEARCH USE ONLY

(PR64075)

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I. Introduction

The Luminescent β -galactosidase assay can be used to verify and quantify β -galactosidase activity in two-hybrid assays. The two-hybrid system is a yeast-based genetic assay to screen for interacting proteins. CLONTECH's MATCHMAKER Two-Hybrid Systems are based on the functional restoration of the physically discrete DNA binding domain (DNA-BD) and transcriptional activation domain (AD) of the yeast GAL4 protein. A known protein is fused to the GAL4 DNA-BD, and a candidate interaction protein or library of proteins is fused to the GAL4 AD. An interaction between the proteins tethers the DNA-BD and AD, resulting in activation of the *lacZ* and *HIS3* reporter genes. The *HIS3* reporter provides a growth selection, and the *lacZ* reporter provides a colorimetric confirmation of identified positives. After His⁺, lacZ⁺ colonies are identified in a two-hybrid screening, the Luminescent β -galactosidase Detection Kit II can be used for sensitive, quantitative measurement in liquid β -galactosidase assays.

Please see the main Luminescent β -galactosidase Detection Kit II User Manual (PT2106-1) for general information, including list of components, about the kit. See one of the MATCHMAKER User Manuals for information about additional materials required, including YPD medium, SD medium, dropout solutions, and Z buffer.

II. Chemiluminescent β -galactosidase Protocols

PLEASE READ ENTIRE PROTOCOL BEFORE BEGINNING

A. Preparation of yeast extracts

1. Inoculate a yeast colony into 5 ml of appropriate medium.

Examples: Inoculate HF7c into SD/-Leu/-Trp/-His.
Inoculate SFY526 into SD/-Leu/-Trp.

2. Incubate at 30°C overnight with shaking (250 rpm).
3. Determine the OD₆₀₀ of the culture. The OD₆₀₀ should be 0.5–1.0.
4. Inoculate ≥ 2 ml of the overnight culture into ≤ 8 ml of YPD liquid medium. Adjust the amounts of overnight culture and YPD medium so that the OD₆₀₀ is approximately 0.2.
5. Incubate culture at 30°C for 3–5 hr with shaking (250 rpm) until the OD₆₀₀ reaches 0.4–0.6.
6. Vortex the tube for 1 min to disperse the cells. Immediately determine the OD₆₀₀.
7. Transfer 1.5 ml of the culture to a separate tube. Centrifuge at 14,000 rpm for 30 sec.
Note: If the OD₆₀₀ in Step 6 is less than 0.4, transfer >1.5 ml of the culture.
8. Carefully remove the supernatant. Add 1.5 ml of Z buffer to the tube, and resuspend the pellet.
9. Centrifuge at 14,000 rpm for 30 sec.
10. Remove the supernatant. Resuspend cells in approximately 300 μ l of Z buffer.
Note: The resuspended cells should have an OD₆₀₀ of approximately 2.5. If the cell density is lower, repeat Steps 7–10, except resuspend the cells in <300 μ l of Z buffer.
11. Vortex the cell suspension. Transfer 100 μ l into a fresh tube.
Note: The remaining suspension can be stored at 4°C or -20°C.
12. Place the tube in liquid nitrogen until the cells are frozen.
13. Thaw the cells in a 37°C water bath for 30–60 sec.
14. Repeat the freeze/thaw cycle once.
15. Spin for 5 min at maximum speed in a refrigerated microcentrifuge.
16. Transfer the supernatant to a fresh tube and keep on ice. Proceed to Section C for chemiluminescent detection of β -galactosidase.

B. Procedure for Single Yeast Colony

Note: This procedure produces qualitative, rather than quantitative, results.

1. Use a sterile 200- μ l pipette tip to pick a single yeast colony (or several colonies) from the plate. Choose a large (>1 mm) colony, if possible. Place the colony in 50 μ l of Z buffer.
2. Resuspend the colony by pipeting up and down several times.
3. Place tube in liquid nitrogen until the cells are frozen.

II. Chemiluminescent β -galactosidase Protocols *continued*

4. Thaw the cells in a 37°C water bath for 30–60 sec.
5. Repeat the freeze/thaw cycle once.
6. Spin for 5 min at maximum speed in a refrigerated microcentrifuge.
7. Transfer the supernatant to a fresh tube and keep on ice. Proceed to Section C for chemiluminescent detection of β -galactosidase.

C. Chemiluminescent detection of β -galactosidase

1. General concerns

It is important to stay within the linear range of the assay. High intensity light signals can saturate the photomultiplier tube in luminometers, resulting in false low readings. In addition, low intensity signals that are near background levels may be outside the linear range of the assay. See the Troubleshooting Guide (Section III) for more information on determining the linear range and adjusting the amount of lysate used to bring the signal within the linear range. Figure 1 in the Troubleshooting Guide gives an example of linear range determination.

2. Chemiluminescent β -galactosidase assay

- a. Warm to room temperature enough reaction buffer for the entire experiment.
- b. Aliquot 20–30 μ l of yeast extract into sample tubes (or into wells of an opaque 96-well, flat-bottom microtiter plate).

Note: The amount of yeast extract required may vary depending upon the amount of expression and the detection device used. Use 10–30 μ l of extract for positive controls and 20–30 μ l of extract for experimental conditions with potentially low levels of β -galactosidase. It is important to vary the amount of extract to keep the signal within the linear range of the assay. Remember to correct for individual sample volumes when tabulating final results.

- c. Add 200 μ l of reaction buffer to each cell lysate and mix gently.
- d. Incubate at room temperature (20–25°C) for 60 min.

Note: Light signals produced during this incubation are stable for >1 hr; therefore, detection can be performed 0–60 min after the incubation.

3. Chemiluminescence detection methods

- a. Detection using a tube luminometer

If the assay is performed in a tube suitable for luminometer readings, the sample may be placed directly in the instrument after Step 2.d. If the assay is not performed in a suitable tube, transfer the entire solution from Step 2.d to an appropriate luminometer tube and place in the instrument. Record light emission as 5-sec integrals.

- b. Detection using a plate luminometer

The entire β -galactosidase assay (Steps 2.a–d) may be performed in white 96-well flat-bottom microtiter plates suitable for plate

II. Chemiluminescent β -galactosidase Protocols *continued*

luminometers. After Step 2.d, simply record light signals as 5-sec integrals.

c. Detection using a scintillation counter

1. Transfer the entire solution from Step 2.d to a 0.5-ml Eppendorf tube.

Note: We highly recommend using scintillation counter adapters which are designed to keep samples upright.

2. Place the tube into the washer of the scintillation counter adapter; place the adapter in the scintillation rack. Set the integration time for at least 15 sec.

Note: Integration times less than 15 sec may not produce very accurate results.

3. To detect chemiluminescent signals, use a single photon count program on the scintillation counter. Consult your scintillation counter manufacturer for further information about photon single count software.

d. Detection by exposure of x-ray film

Light emission can also be recorded by exposure of x-ray film to opaque 96-well flat-bottom microtiter plates. The resulting spots on the film can be quantitated by comparison to positive and negative control incubations (see Section D below). Note that x-ray film is several orders of magnitude less sensitive than a luminometer or scintillation counter.

Overlay the microtiter plate with x-ray film, cover the film with plastic wrap, and place a heavy object such as a book on top to hold the film in place. Expose the film at room temperature for 5–30 min.

Note: To compare samples accurately, you must remain within the linear response capability of the x-ray film. We therefore recommend that you obtain several different exposures.

D. Proper Use of Controls

1. Negative controls

A negative control is necessary to determine the level of background signals associated with the cell lysates. We suggest using yeast lysate from cells not expressing β -galactosidase as a negative control. The averaged value obtained for three negative controls should be subtracted from each experimental result.

2. Positive controls

a. Positive control for method of detection

The positive control bacterial β -galactosidase can be used to confirm that the detection method is working. To do this, simply add 1–2 μ l of the positive control bacterial β -galactosidase to cell lysates obtained from yeast cells not expressing β -galactosidase. This should give a strong positive signal.

II. Chemiluminescent β -galactosidase Protocols *continued*

A dilution series of the positive control enzyme can also be used to determine the linear range of the assay.

b. MATCHMAKER positive controls

All MATCHMAKER Systems are provided with positive control experiments. We recommend that you use them in a β -galactosidase assay.

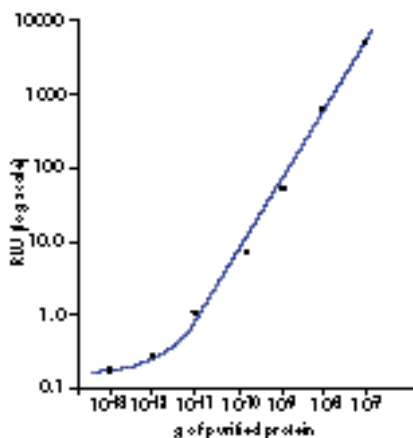
III. Troubleshooting Guide

A. Determining linear range of the assay

If in doubt about the linear range of the assay, prepare and assay a dilution series using the positive control bacterial β -galactosidase provided with the kit. In the example shown in Figure 1, light readings from approximately 2 to 6,000 RLU are within the linear range of the assay. In this example, the linear range corresponds to approximately 10^{-12} – 10^{-7} g of the positive control β -galactosidase or approximately 10^{-6} – 10^{-1} units of β -galactosidase activity.

Note: Figure 1 is offered only as an example. The linear range should be independently determined for each luminometer and each set of experimental conditions.

Figure 1: Determining the linear range of the assay. Serial dilutions of the positive control β -galactosidase were prepared and assayed as described in the protocol using a Turner Model TD-20e luminometer with a 5-sec integration time. Background signals from samples containing no β -galactosidase have been subtracted from all values. RLU = Relative Light Units.



B. Little or no signal from yeast extracts

There are several possible explanations for a weak or nondetectable signal in the chemiluminescence assay.

1. To ensure that your detection method is working, use the bacterial β -galactosidase provided with the kit as a positive control (as described in Section II.D.2).
2. Make sure that you use liquid nitrogen for the freeze/thaw cycles. Dry ice/EtOH baths or -70°C freezers can result in inadequate lysis.
3. If the signal is still weak, try the following modifications to increase the signal:
 - a. Increase the quantity of yeast cells used in extract preparation
 - b. If background signals obtained from negative controls are low, then the volume of yeast extracts may be increased.
 - c. If using a tube or plate luminometer to monitor chemiluminescence,

III. Troubleshooting Guide *continued*

refer to the instrument instructions for means of increasing the sensitivity of light detection.

- d. If using exposure of x-ray film to monitor chemiluminescence, increase the exposure time.

C. Excessive background signal

If the signal from experimental samples is sufficiently high, decrease the amount of yeast extract assayed at Step II.C.2.b or dilute the yeast extract using 1X lysate buffer.

D. Signal exceeds linear range of the assay

This problem is easily corrected either by assaying a lower volume of cell lysate at Step II.C.2.b or by diluting the samples.

IV. References

1. Bartel, P. L., Chien, C.-T., Sternglanz, R. & Fields, S. (1993) Using the two-hybrid system to detect protein-protein interactions. In *Cellular Interactions in Development: A Practical Approach*, ed. Hartley, D. A. (Oxford University Press, Oxford), pp. 153–179.
2. Chien, C. T., Bartel, P. L., Sternglanz, R. & Fields, S. (1991) The two-hybrid system: A method to identify an dclone genes for proteins that interact with a protein of interest. *Proc. Natl. Acad. Sci. USA* **88**:9578–9582.
3. Fields, S. & Song, O. (1989) A novel genetic system to detect protein-protein interactions. *Nature* **340**:245–247.

V. Related Products

Product	Cat. #	Size
MATCHMAKER Two-Hybrid System 2	K1604-1	each
MATCHMAKER Two-Hybrid System	K1605-1	each
MATCHMAKER One-Hybrid System	K1603-1	each
TransAct™ Assay Kit	K1608-1	each
YEASTMAKER™ Yeast Transformation System	K1606-1	each
Mammalian MATCHMAKER Two-Hybrid Assay Kit	K1602-1	each

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