P01002

HTX Kit

High Throughput β-Galactosidase Assay

Scope

The HTX kit allows simultaneous quantitative determination of β-galactosidase activity of multiple samples with a minimum of hands-on time.

Applications include measurement of β-galactosidase activity of yeast clones in yeast two-hybrid or DUALmembrane screens, quantification of the strength of interaction of defined protein pairs in yeast two-hybrid or DUALmembrane assays, as well as measurement of β-galactosidase reporter gene activity in yeast or bacterial cells.

Contents and storage

Upon receipt of the kit, please make sure to store the individual components as indicated below.

HTX assay kit

One-Step Lysis and Assay Reagent
- Contains buffer salts and a mixture of mild detergents
- 50 ml (sufficient for 4 x 96 assays)
- Store at room temperature

Dye stock solution
- Contains X-Gal substrate and buffer salts
- 250 µl
- Store at -20° C and protect from light

Materials to be supplied by the user

- β-Mercaptoethanol
- 96 deep-well blocks or snap-cap tubes
- 96 well microtiter plate (we recommend clear flat bottom plates for optimal results)
- Appropriate SD liquid medium

Before first use of the HTX kit
- Add 25 µl β-Mercaptoethanol to the One-Step lysis and assay reagent, shake well to mix

Introduction

Measuring β-galactosidase activity is an important step in every yeast two-hybrid assay. Most yeast two-hybrid systems take advantage of the *Escherichia coli lacZ* gene encoding β-galactosidase (β-gal) as a colorimetric reporter gene. The use of a β-gal reporter gene complements growth selection and offers an easy means to quantify the interaction strengths of clones isolated in a yeast two-hybrid screen (Fields and Song 1989; Fashena *et al*., 2000).

Over the years, many methods have been developed to measure β-gal activity in yeast two-hybrid and related genetic screening systems (Serbriiskii and Golemis, 2000). However, most commonly used methods are only qualitative, time-consuming and cumbersome when processing large numbers of samples.

The HTX assay has several advantages: it allows simultaneous quantitative measurements of large numbers of samples with a minimum of hands-on time, and it requires only standard equipment available in any laboratory. The minimal number of handling steps involved is especially advanatgeous when hundreds of different samples have to be processed, for example in the context of a yeast two-hybrid screen. Figure 1 gives an overview of the HTX procedure.

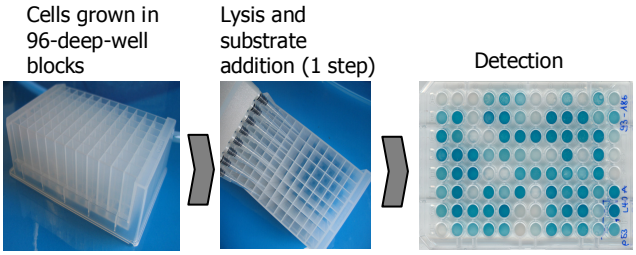

Figure 1. Yeast cultures are grown in a 96 deep-well block until they have reached the late exponential phase and pelleted by centrifugation. The pellets are lysed by addition of the one-step lysis/substrate buffer and transferred to a clear-bottom 96 well plate. Color development over time is monitored by eye and recorded using either a regular flatbed scanner or a spectrophotometer.

Results can be recorded either using a simple flatbed scanner or a microplate reader. When using a flatbed scanner, the color intensity of individual wells can be measured and quantified using using software programs such as the free NIH image package (available from http://rsb.info.nih.gov/nih-image/Default.html). When using a plate reader, individual measurements are best processed using the software distributed with the instrument.

This manual provides two protocols:

- Protocol 1 describes a quick and easy method, suitable for the semi-quantitative determination of β-gal levels. This method is best suited for measuring a large number of samples at the same time, for instance when analysing primary clones isolated in a yeast two-hybrid screen. The semi-quantitative measurements help the user to select the strongest interactors for further analysis.

- Protocol 2 describes a protocol for simultaneously measuring the exact β-gal levels of a group of samples, for instance when comparing the interaction strength of several protein pairs in a

yeast two-hybrid assay. When compared to Protocol 1, the method requires several additional steps, but has the advantage that measurements are quantitative and that comparison of •-gal levels derived from different experiments is possible.

Protocol 1: Semi-quantitative method

Summary

Yeast cultures are grown to late exponential phase, pelleted and directly resuspended in lysis/substrate buffer. Blue color development is monitored and recorded using either a flatbed scanner or a microplate reader.

Duration

20 min hands-on time, 30 min to several hours for color development.

Protocol

1. Preparing the yeast culture: For each interaction pair, pick several yeast colonies from selection plate and inoculate into a snap-cap tube containing 1 ml of the appropriate selective medium.

   Note
   We recommend that selection is applied only to maintain the plasmids encoding the interacting proteins, and that no selection is applied for interaction-dependent growth (e.g. when using Dualsystems DUALhybrid or DUALmembrane systems, use SD medium lacking tryptophan and leucine to select for bait and prey plasmids only, as opposed to using SD medium lacking tryptophan,leucine and histdine, which would additionally select for the protein-protein interaction. In our experience, applying selective pressure only to the plasmids guarantees more reproducible results.

   When processing large numbers of samples (e.g. more than 50 samples), inoculate into 2 ml deep-well plates containing 1 ml of the appropriate selective medium. Grow at 30°C with shaking (250 rpm), until the culture has reached an OD_{546} of 0.5-0.8.

2. Centrifuge at 2000 x g for 5 minutes
3. Discard the supernatant
4. Prepare the lysis mixture: To process one 96-well plate, mix 9.95 ml of **One-Step Lysis and Assay Reagent** with 50 µl of **Dye stock solution.**
5. Add 100 µl of lysis mixture to each well and vortex for 30 seconds to 1 minute to resuspend the cells
6. Transfer the resuspended cells to the clear-bottom 96-well microplate supplied in the kit
7. Monitor color development over time and record the plate at different time intervals, using either a flatbed scanner or a microplate reader set to 615 nm.
8. When using a flatbed scanner, the relative levels of blue coloration of each sample can be quantified using NIH image. Please see http://rsb.info.nih.gov for instructions on downloading NIH image and consult the user manual supplied with the NIH software package for instructions on how to quantify measurements.
9. When using a microplate reader, use the software supplied with the instrument for quantification.

Protocol 2: Quantitative method

Summary

Yeast cultures are pelleted and directly resuspended in lysis/X-gal buffer. Blue color development is monitored over time and quantitated

Duration

30 min hands-on time, 30 min to several hours for color development.

Protocol

1. Culture setup: for each interaction pair, pick several yeast colonies from selection plate and inoculate into a snap-cap tube containing 5 ml of selective medium or into 2 ml deep-well plates containing 1 ml of selective medium. Grow for at 30°C with shaking (250 rpm) until the cultures have reached an approx. OD_{546} of 0.5-0.8.
2. Measure OD_{546} of each culture and remove an aliquot corresponding to 0.5 OD.
3. Centrifuge at 2000 g for 5 min
4. Discard supernatant
5. Prepare lysis mixture: For 10 reactions, mix 995 µl of **One-Step Lysis and Assay Reagent** with 5 µl of **Dye stock solution.**
6. Add 100 µl of lysis mixture to each reaction and vortex
7. Transfer to a 96-well microplate
8. Monitor color development at 615 nm (A_{615}) using a densitometer. Alternatively, a flatbed scanner can be used in combination with the NIH image software package to quantitate color formation.
9. To quantitate β-galactosidase activity, use the following equation:

$$\frac{1000 \times A_{615}}{t \times V \times OD_{546}} = \beta\text{-galactosidase activity}$$

t = incubation time (min)
V = volume of cells used in the assay (ml)

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Troubleshooting

Yeast do not lyse
Do not add more than the equivalent of 1 ml of culture at 1 OD. Perform the test with fresh overnight cultures.

Color does not develop
Double the amount of yeast culture used in the assay.

Make sure X-Gal has been added to One-Step Lysis and Assay Reagent.

Make sure X-Gal has been stored in a dark place at –20°C for no longer than 6 months.

Color development may take up to several hours for weak interactions. Add a strong interactor pair as a positive control.

Image processing

The **NIH image** software package for Macintosh may be downloaded from http://rsb.info.nih.gov/nih-image/

The **Scion image** software package for PC may be downloaded from www.scioncorp.com

References

Fashena S. J., Serebriiskii I., Golemis E. A. (2000) The continued evolution of two-hybrid screening approaches in yeast: how to outwit different preys with different baits. Gene 250:1-14.

Fields S., Song O. (1989) A novel genetic system to detect protein-protein interactions. Nature 340:245-246.

Serebriiskii, I. G. and Golemis, E. A. (2000) Uses of *lacZ* to study gene function: evaluation of β-galactosidase assays employed in the yeast two-hybrid system. Anal. Biochem. 285:1-15.

Related products

DUALmembrane starter kits (P012-P014)
DUALmembrane pairwise interaction kit (P01501)
DUALhybrid kit (P01004)
DUALhunter kit (P01005)
DS Yeast transformation kit (P01003)

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