



## Product Sheet

# Estrogen Disruptor Screening Kit

### Overview

The estrogen disruptor screening kit contains two proprietary *Saccharomyces cerevisiae* strains genetically programmed to produce bioluminescent signals without the need for substrate addition or investigator interaction. Simply expose the strains to your compounds of interest and determine both estrogenicity and cytotoxicity in one simple package!

### Contents

*Saccharomyces cerevisiae* strain BLYR  
*Saccharomyces cerevisiae* strain BLYES

### Required Materials Supplied By User

- Methanol
- 17β-estradiol
- 96 well microtiter plate
- Spectrophotometer
- Bioluminescent plate reader

### Safety

These organisms are classified as biosafety level 1, however, appropriate precautions should be taken when working with any organism. Please consult with your institutional safety office to determine the appropriate personal protective equipment required for use. At a minimum, protective gloves and eye protection should be worn when handling or working with these organisms.

### Disclaimers

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490 BioTech  
2450 E.J. Chapman Drive  
Knoxville, TN 37996  
[www.490BioTech.com](http://www.490BioTech.com)



## Product Details

### *Saccharomyces cerevisiae* BLYR

This strain has been engineered to produce a continuous bioluminescent signal. Because the production of bioluminescence is dependent on cellular health, this strain acts as a control for cytotoxicity, with decreasing bioluminescent output indicative of increasing toxicity.

### *Saccharomyces cerevisiae* BLYES

This strain is engineered to produce a bioluminescent signal only in response to estrogen disruptor compound detection. Binding of an activated human estrogen receptor to upstream estrogen response elements triggers activation of the bioluminescent genes. This strain is used for detection and quantification of estrogen disruptor compounds.

## Growth Conditions

Upon arrival, frozen cells should be thawed immediately or stored at or below  $-80^{\circ}\text{C}$ .

**Failure to immediately thaw or properly store cells will result in cell death.** Cells obtained as active colonies should be immediately subcultured into fresh medium to ensure active growth and then used directly for assay development. Repeated liquid subculture is not recommended and can lead to decreased activity.

To thaw frozen cells for use, place the frozen vial into a pre-warmed  $55^{\circ}\text{C}$  water bath at a level capable of covering the cell mixture. **Do not fully submerge the vial; this could lead to contamination of the culture.** Hold cells stationary in the water bath (do not shake) just until liquid begins to thaw. Using aseptic technique, immediately transfer the cells to an appropriate liquid or solid medium and grow at the recommended temperature.

Cultures should be grown on chemically defined media **without leucine, uracil, and tryptophan**. The optimal growth temperature for the BLYR and BLYES strains is  $30^{\circ}\text{C}$ .

## Sample Estrogen Disruptor Detection Protocol

### 1. Before You Begin

Sterilize all glassware by baking at  $400^{\circ}\text{C}$  for 4 hours. Alternatively, glassware can be sterilized by baking at  $250^{\circ}\text{C}$  for 8 hours. **The use of sterilized glassware is highly recommended, as plasticware can leach estrogenic chemicals during use, leading to false positives.**

Prepare *S. cerevisiae* strains BLYR and BLYES by growing overnight in 30 ml volumes of medium in baked 250 ml Erlenmeyer flasks at  $30^{\circ}\text{C}$  and 200 rpm shaking to an approximate optical density at 600nm ( $\text{OD}_{600}$ ) of 0.5 to 0.8. For environmental samples, centrifuge cultured yeast cells and resuspend in fresh 2x media to an  $\text{OD}_{600}$  of 2.0 to increase potential toxicity tolerance.

### 2. Preparation Of A Standard Curve/Positive Control

17β-estradiol (supplied by the user) should be used as a positive control for the detection of estrogenicity and for the generation of a standard curve. All 17β-estradiol samples should be diluted in methanol to ensure proper solubility.

Prepare a fresh  $1.0 \times 10^{-2}$  M solution of DHT in methanol. Make serial dilutions as shown in Table 1, and transfer 20 μl of each dilution into the appropriate well of a 96 well microtiter plate. Allow the methanol to dry by evaporation.

Add 200 μl aliquots of the BLYES strain to each of these wells.

**Table 1.** Serial dilution concentrations (molar concentrations) of solution added to the standard curve wells. 20 μl of each of these solutions is added to the wells of the microtiter plate, methanol is then evaporated and 200 μl of BLYES cells are added to each well. Note that after addition of BLYES final molar concentrations will range from  $1 \times 10^{-7}$  to  $2.5 \times 10^{-13}$ .

$1 \times 10^{-6}$	$1 \times 10^{-7}$	$1 \times 10^{-8}$	$1 \times 10^{-9}$	$1 \times 10^{-10}$	$1 \times 10^{-11}$
$5 \times 10^{-7}$	$5 \times 10^{-8}$	$5 \times 10^{-9}$	$5 \times 10^{-10}$	$5 \times 10^{-11}$	$5 \times 10^{-12}$
$2.5 \times 10^{-7}$	$2.5 \times 10^{-8}$	$2.5 \times 10^{-9}$	$2.5 \times 10^{-10}$	$2.5 \times 10^{-11}$	$2.5 \times 10^{-12}$

## Testing Specific Chemicals For Estrogenicity

To test the estrogenicity of a specific chemical, prepare a serial dilution of the chemical in methanol as described in Table 1. Carefully introduce 20 µl of each concentration into the appropriate well of a 96 well microtiter plate. Allow any residual methanol from this procedure to dry by evaporation. Alternatively, dimethyl sulfoxide (DMSO) can be used as a solvent if the chemical of interest is not soluble in methanol. The final concentration of DMSO should not exceed 1% (i.e., add no more than 2 µl DMSO to 200 µl culture) to minimize solvent toxicity.

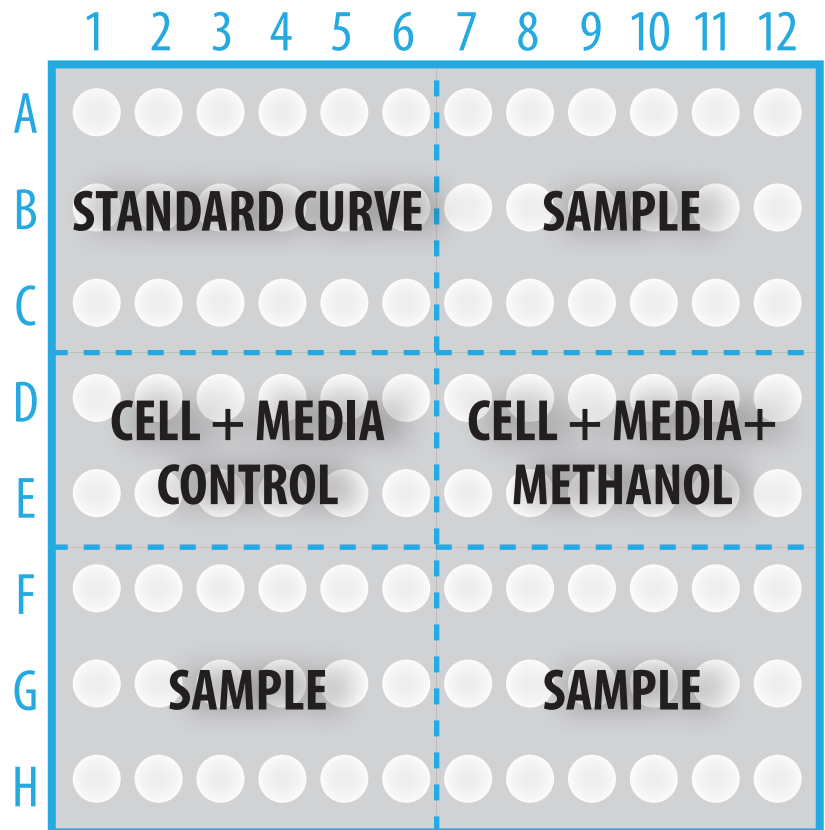
Place 200 µl aliquots of the BLYES strain into each of these wells. Measure the resultant bioluminescent readings at appropriate intervals (every 60 min for 12 hours is typical) using a plate reader.

**Figure 1**

Typical layout for conducting an estrogenicity assay against specific chemicals in a 96 well plate.

### Notes

Figure 1 shows the plate map of a typical estrogen disruptor detection assay against specific chemicals. For each plate, one standard and three test chemicals can be tested. To test additional samples, prepare more plates as needed. It is highly recommended that negative controls consisting of wells with (1) only growth medium and cells and (2) wells with only growth medium, cells, and evaporated methanol (or DMSO) are also included with each run.



## Testing Water Samples For Estrogenicity

For testing water samples, the same standard curve/positive control and negative controls described in *Testing Specific Chemicals For Estrogenicity* should be used.

Prior to testing, all water samples should be concentrated to 2000x by solid phase extraction according to EPA Method 3535/3535A. Serial dilutions should then be prepared to generate 2000x – 0.005x plating concentrations, which will give final concentrations ranging from 1000x to 0.0025x as shown in Table 2.

Add 100 µl of each water sample to the plate as shown in Figure 2.

Add 100 µl aliquots of strain BLYES to each well. For environmental sample testing it is recommended that each BLYES aliquot be at an OD<sub>600</sub> of 2.0.

**Table 2.** Final concentrations of water samples following addition of 100 µl aliquots of strain BLYES to samples.

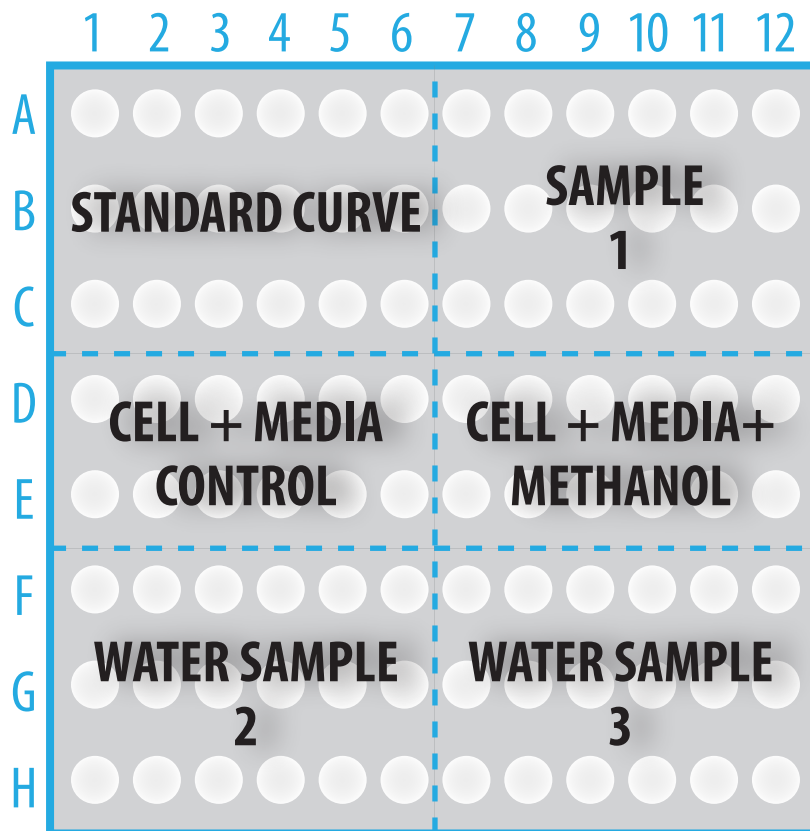
1000x	100x	10x	1x	0.1x	0.01x
500x	50x	5x	0.5x	0.05x	0.005x
250x	25x	2.5x	0.25x	0.025x	0.0025x

**Figure 2**

Typical layout for conducting an estrogenicity assay against water samples in a 96 well plate.

**Notes**

Figure 2 shows the plate map of a typical estrogen disruptor detection assay against water samples. For each plate, one standard and three water samples can be tested. To test additional samples, prepare more plates as needed. It is highly recommended that negative controls consisting of wells with (1) only growth medium and cells and (2) wells with only growth medium, cells, and evaporated methanol (or DMSO) are also included with each run.



**Testing Cytotoxicity**

If potential sample cytotoxicity is a concern, dilution platings for that sample should be performed in duplicate. One set of dilutions should be mixed with aliquots of strain BYLES as described in *Testing Specific Chemicals For Estrogenicity* or *Testing Water Samples For Estrogenicity* above. The second set of dilutions should be treated with similarly prepared aliquots of strain BLYR. Decreases in bioluminescent output from strain BLYR indicate the presence of cytotoxic effects.

A typical dual estrogenicity/cytotoxicity assay is shown in Figure 3.

**Figure 3**

Example of a dual estrogenicity/cytotoxicity assay.

