Product Sheet

Estrogen/Androgen Disruptor Screening Kit

Overview

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

Contents

Saccharomyces cerevisiae strain BLYR
Saccharomyces cerevisiae strain BLYES
Saccharomyces cerevisiae strain BLYAS

Required Materials Supplied By User

- Methanol
- 17β-estradiol
- 5α-dihydrotestosterone (DHT)
- 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

This product is for research use only. It is not intended for human or diagnostic use. The user is ultimately responsible for the safe storage, handling, and use of this product. 490 BioTech is not liable for any injuries or damages resulting from receipt and/or use of this product.

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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.

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

Growth Conditions

Upon arrival, frozen cells should be thawed immediately or stored at or below –80°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°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, BLYES, and BLYAS strains is 30°C.

Before You Begin

Sterilize all glassware by baking at 400°C for 4 hours. Alternatively, glassware can be sterilized by baking at 250°C for 8 hours. **The use of sterilized glassware is highly recommended, as plasticware can leach estrogenic and endrogenic chemicals during use, leading to false positives.**

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

Preparation Of A Standard Curve/Positive Control

For Estrogenicity Screening
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 1.0×10^{-2} M solution of 17β-estradiol 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.

For Androgenicity Screening

5α-dihydrotestosterone (DHT) (supplied by the user) should be used as a positive control for the detection of androgenicity and for the generation of a standard curve. All DHT samples should be diluted in methanol to ensure proper solubility.

Prepare a fresh 1.0×10^{-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 BLYAS 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 or BLYAS cells are added to each well. Note that after addition of BLYES or BLYAS the final molar concentrations will range from 1×10^{-7} to 2.5×10^{-13}.

1×10^{-6}	1×10^{-7}	1×10^{-8}	1×10^{-9}	1×10^{-10}	1×10^{-11}
5×10^{-7}	5×10^{-8}	5×10^{-9}	5×10^{-10}	5×10^{-11}	5×10^{-12}
2.5×10^{-7}	2.5×10^{-8}	2.5×10^{-9}	2.5×10^{-10}	2.5×10^{-11}	2.5×10^{-12}

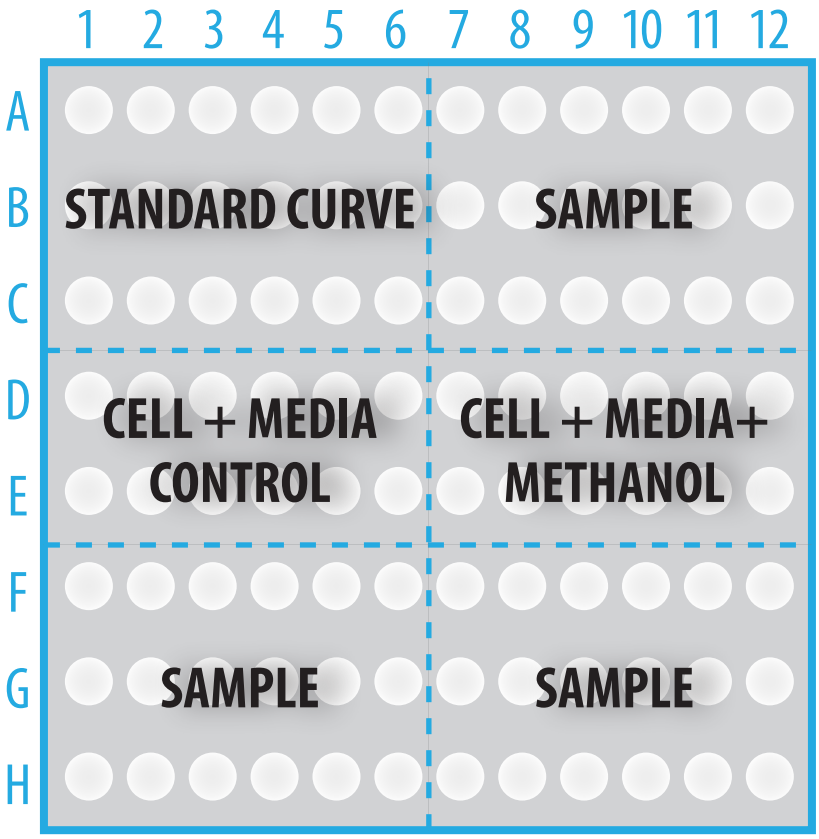
Testing Specific Chemicals For Estrogenicity

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/androgenicity assay against specific chemicals in a 96 well plate.



Notes

Figure 1 shows the plate map of a typical estrogen/androgen disruptor detection assay. 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_{600} of 2.0.

Table 2. Final concentrations of water samples following addition of 100 µl aliquots of strain BLYES or BLYAS 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

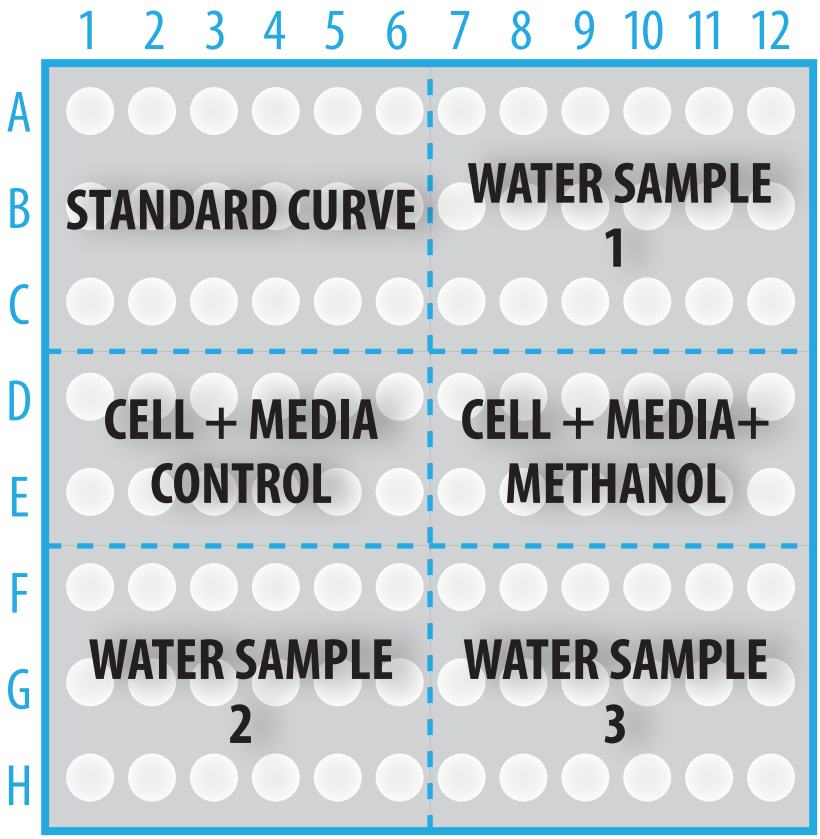


Figure 2
Typical layout for conducting an estrogenicity/androgenicity assay against water samples in a 96 well plate.

Testing Specific Chemicals For Androgenicity

To test the androgenicity 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 BLYAS 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.

Notes

Figure 1 shows the plate map of a typical androgen 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 Androgenicity

For testing water samples, the same standard curve/positive control and negative controls described in *Testing Specific Chemicals For Androgenicity* 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 BLYAS to each well. For environmental sample testing it is recommended that each BLYAS aliquot be at an OD_{600} of 2.0.

Notes

Figure 2 shows the plate map of a typical androgen 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 or BLYAS as described in *Testing Specific Chemicals For Estrogenicity/Androgenicity* or *Testing Water Samples For Estrogenicity/Androgenicity* 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 or androgenicity/cytotoxicity assay is shown in Figure 3.

Figure 3
Example of a dual estrogenicity or androgenicity/ cytotoxicity assay.