Yeast Estrogen Screen (YES-assay) for the detection of ER-active compounds

**Note:** Protocol and the yeast is a gift of Prof. J.P. Sumpter, Brunel University, UK

### I. PREPARATION AND STORAGE OF MINIMAL MEDIUM AND MEDIUM COMPONENTS

Minimal medium and medium components prepared in glassware contaminated with an estrogenic chemical will lead to elevated background expression. Glassware, spatulas, stirring bars, etc., must be scrupulously cleaned, and should not have had prior contact with steroids. Rinse glassware, spatulas, stirring bars twice with absolute ethanol, and leave to dry. Alternatively, wash twice with methanol, and once with ethanol.

#### Chemicals

**Minimal Medium pH 7.1**

Add 13.61 g KH2PO4, 1.98 g (NH4)2S04, 4.2 g KOH pellets, 0.2 g MgSO4, 1 ml Fe2(SO4)3 solution (40 mg/50 ml H2O), 50 mg L-leucine, 50 mg L-histidine, 50 mg adenine, 20 mg L-arginine-HCl, 20 mg L-methionine, 30 mg L-tyrosine, 30 mg L-isoleucine, 30 mg L-lysine-HCl, 25 mg L-phenylalanine, 100 mg L-glutamic acid, 150 mg L-valine, and 375 mg L-serine to 1 L double-distilled water. Place on heated stirrer to dissolve.

Dispense 45 ml aliquots into glass bottles.

Sterilise at 121°C for 10 min, and store at room temperature.

**D-(+)-Glucose**

Prepare a 20% w/v solution.

Sterilise in 20 ml aliquots at 121°C for 10 min. Store at room temperature.

**L-Aspartic Acid**

Make a stock solution of 4 mg/ml.

Sterilise in 20 ml aliquots at 121°C for 10 min. Store at room temperature.

**Vitamin Solution**

Add 8 mg thiamine, 8 mg pyridoxine, 8 mg pantothenic acid, 40 mg inositol, and 20 ml biotin solution (2 mg/l/100 ml H2O) to 180 ml double-distilled water.

Sterilise by filtering through a 0.2 um pore size disposable filter, in a laminar air flow cabinet.

Filter into sterile glass bottles in 10 ml aliquots.

Store at 4 °C.

**L-Threonine**

Prepare a solution of 24 mg/ml.

Sterilise in 10 ml aliquots at 121°C for 10 min. Store at 4 °C.

**Copper (II) Sulfate**

Prepare a 20 mM solution.

Sterilise by filtering through a 0.2 um pore size filter, in a laminar flow cabinet. Filter into sterile glass bottles in 5 ml aliquots.

Store at room temperature.

**Chlorophenol red-β-D-galactopyranoside (CPRG)**

Make a 10 mg/ml stock solution. Sterilise by filtering through a 0.2 um pore size filter into sterile glass bottles, in a laminar flow cabinet.

Store at 4 °C.
II. **PREPARATION AND STORAGE OF CHEMICALS**

Glassware must be scrupulously cleaned since contamination may give rise to false positives. Rinse all glass bottles twice with absolute ethanol (or twice with methanol, and once with ethanol), and leave to dry. Weigh chemicals directly into bottles. Prepare the 17β-estradiol stock for the standard curve in absolute ethanol, at 2xl0^-7 M (54.48 ug/L). Stock solutions of tested chemicals are usually prepared in absolute ethanol or DMSO. Environmental samples are usually prepared in DMSO.

III. **ASSAY PROCEDURE**

*Note: Carry out all yeast work aseptically in a laminar flow cabinet.*

**Day 0**
*Preparation of yeast for assay:*
Prepare growth medium by adding 5 ml glucose solution, 1.25 ml L-aspartic acid solution, 0.5 ml vitamin solution, 0.4 ml L-threonine solution, and 125 ul copper (II) sulfate solution to 45 ml minimal medium. Transfer to a sterile conical flask (final volume of approximately 50 ml). Add 125 ul of 10X concentrated yeast stock from cryogenic vial. Incubate at 28°C for approximately 24 hours on an orbital shaker, or until turbid.

**Day 1**
Serially dilute chemicals in absolute ethanol or DMSO (100 ul volumes), in a 96-well microtitre plate. Transfer 10 ul aliquots of each concentration (for EtOH) or 4 ul for DMSO to a 96-well optically flat bottom microtitre plate. Add 10 ul absolute ethanol or 4 ul DMSO to blank wells. When used EtOH leave chemicals in the assay plate to evaporate to dryness.

Prepare assay medium by adding 0.5 ml CPRG to 50 ml fresh growth medium. Seed this medium with 4x10^7 yeast cells (usually between 0.5 ml and 2 ml) from the 24-h culture prepared on Day 0. For every 2.5 assay plates, prepare 50 ml assay medium. Add 200 ul of the seeded assay medium (growth medium containing CPRG and yeast) to well using a multichannel pipette.

Each plate should contain at least one row of blanks (solvent and assay medium only), and each assay should have a 17B-estradiol standard curve.

Be aware that same chemicals (particularly alkylphenolic chemicals) creep across the assay plate to contaminate adjacent wells, and should therefore be placed on separate plates.

Seal the plates with autoclave tape and shake vigorously for 2 min on a titre plate shaker. Incubate at 32°C in a naturally ventilated heating cabinet.

**Day 2**
Shake the plates vigorously for 2 min, to mix and disperse the growing cells. Return to 32°C incubator.

**Day 4**
After incubating for 3 days, shake plates (2 min) and leave for approximately 1 hour to allow the yeast to settle. Read the plates at an absorbance of 540 nm (optimum absorbance for CPRG 575 nm) and 620 nm (for turbidity) using a plate reader.
IV. CALCULATIONS

To correct for turbidity the following equation needs to be applied to the data:
Corrected value = chem. abs. (540 nm) - [chem. abs. (620 nm)-blank abs. (620 nm)]

To determine the estrogenic potency of an environmental extract, the response of a suitable concentration can be interpolated in a dose-response curve of the reference compound 17β-estradiol (E2). A suitable concentration is a concentration giving a response that fits into the linear part of the E2 dose-response curve. The estrogenic potency of the compound or environmental extract is expressed as EEQ (estradiol equivalents). The estrogenic potency of a compound is usually expressed relative to E2 using the EC50 values.

V. KEY REFERENCES


