Appendix B4

Protocol for Yeast-Based Androgen Receptor Assay

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Yeast-Based Androgen Receptor Assay

1. MATERIALS:

a. Yeast Nitrogen Base without Amino Acids
b. D-(+)-Glucose (Dextrose)
c. Copper Sulfate Pentahydrate
d. 2-Mercaptoethanol
e. Oxalyticase, Enzogenetics, Corvallis, OR, USA, catalog# 0-105, 5 mg
f. Glycerol
g. Di-sodium phosphate (Na_2HPO_4)
h. Monosodium phosphate (NaH_2PO_4)
i. Potassium Chloride (KCl)
j. Magnesium Sulfate ($MgSO_4$)
k. 0-Nitrophenyl ß-D-Galacto-Pyranoside (ONGP)
l. Lauryl Sulfate (Sodium dodecyl sulfate)
m. Sodium Chloride (NaCl)
n. L-Lysine-HCl
o. Adenine Sulfate
p. L-Tryptophan
q. Uracil

2. EQUIPMENT:

a. Microplate reader with kinetics capability using 590 and 420 nm filters
b. Multi-channel pipetter
c. Graduated cylinders, 100, 500, and 1000 ml
d. Balance
e. Stir plate
f. Magnetic stir bars
g. 1-100 µl pipetter
h. 1-10 µl pipetter
i. 50 ml centrifuge tube racks
j. Spectrophotometer with a 600 nm filter
k. pH meter
l. Beakers, 1000 ml
m. 30°C incubator with ability to shake 300 rpm
n. Pipette aid
o. Autoclave
p. Culture flask, 125 ml

3. SUPPLIES:

a. 1-100 µl pipette tips
b. 1-10 µl pipette tips
c. Multi-channel pipette reservoirs
d. 96 well plate
e. 50 ml centrifuge tubes, polypropylene, sterile
f. 100, 500, and 1000 ml glass bottles, with screw cap, sterile
g. 100, 500, and 1000 ml-0.2 µ filter units for sterilization
h. 1.5 ml semi-micro cuvettes
i. 1, 2, 5, 10, 25 ml pipettes
j. Weigh boats
k. 1.5 ml microfuge tube

PREPARATION:

1. **10X Yeast Nitrogen Base without Amino Acids (YNB)**
   a. Weigh out 67g Yeast Nitrogen Base without Amino Acids.
   b. Place in 1000 ml graduated cylinder.
   c. Bring up to 1000 ml with distilled water.
   d. Mix with magnetic stir bar on stir plate.
   e. Filter sterilize with 1000 ml-0.2 µ filter unit. Transfer to 1000 ml sterile glass bottle.

2. **20% Dextrose Stock**
   a. In 1000 ml beaker, dispense 800 ml distilled water, add magnetic stir bar, and place on magnetic stirrer.
   b. Weigh out 200g Dextrose
   c. Add Dextrose slowly to vigorously stirring distilled water.
   Filter sterilize with 1000 ml-0.2 µ filter unit. Transfer to 1000 ml sterile glass bottle.

3. **10 mM Copper Sulfate**
   a. Weigh out 0.25g Copper Sulfate pentahydrate. Place in 100 ml graduated cylinder.
   b. Bring up to 100 ml with distilled water.
   c. Filter sterilize with 100 ml-0.2 µ filter unit. Transfer to 100 ml sterile glass bottle.

4. 10% SDS

a. Weigh out 10g Lauryl Sulfate. Place in 100 ml graduated cylinder.

N. Bring up to 100 ml with distilled water. Mix well.

O. Transfer to 100 ml sterile glass bottle.

5. 1M Sodium Chloride

a. Weigh out 58.44g NaCl. Place in 1000 ml graduated cylinder.

b. Bring to 1000 ml with distilled water. Mix well.

c. Filter sterilize with 1000 ml-0.2 μ filter unit. Transfer to 1000 ml sterile glass bottle.

6. 50% Glycerol with 100mM NaCl

a. Put 50 ml glycerol into 100 ml graduated cylinder.
b. Add 10 ml of 1M NaCl solution.
c. Bring up to 100 ml with distilled water. Mix well.
d. Transfer into 100 ml sterile glass bottle.

7. Oxalyticase

To 5 mg bottle of oxalyticase, add 1.11 ml of 50% Glycerol solution, making a 200U/μl solution. Mix well. Store at 4°C.

8. Z Buffer

a. Weigh out:

16.1 g	Na_2HPO_4
5.5 g	NaH_2PO_4
0.75 g	KCl
0.25 g	$MgSO_2$

b. Place in 1000 ml graduated cylinder.

c. Bring up to 800 ml with distilled water.

d. Adjust pH to 7.0 while stirring with stir bar on stir plate.

e. Bring up to 1000 ml with distilled water.

f. Filter sterilize with 1000 ml-0.2 μ filter unit. Transfer to 1000 ml sterile glass bottle.

9. Amino Acids

a. LYS-1.8g L-lysine-HCl in 500 ml of distilled water. Autoclave.

b. TRP-2.4 g L-tryptophan in 500 ml of distilled water. Filter sterilize with 500 ml-0.2 μ filter unit.

c. URA-1.2 g uracil in 500 ml of distilled water. Autoclave.

d. ADE-0.6 g adenine sulfate in 500 ml of distilled water. Autoclave.

10. Growth Media for AR Transformed Yeast

a. Measure out 50 ml 10X YNB, 50 ml 20% Dextrose, 5 ml Lysine, 5 ml Tryptophan, 5 ml Uracil, and 17 ml Adenine in 500 ml graduated cylinder. Mix well.

b. Bring up to 500 ml with distilled water.

c. Filter sterilize with 500 ml-0.2 μ filter unit. Transfer to 500 ml sterile glass bottle.

ASSAY:

1. Start an overnight culture of androgen receptor transformed yeast in growth media by making a 1:10 dilution of a log-phase culture of yeast.
2. Dilute the overnight culture of yeast in the morning by half in growth media. Start the assay in the afternoon
3. Dilute cells to an OD_{600} of 0.06 in growth media.
4. Add 100 µl 10 mM Copper Sulfate solution/20 ml growth media.
5. Dispense 5 ml diluted yeast solution into a 50 ml **polypropylene** centrifuge tube (1 tube per dose of chemical being tested and 1 tube per dose in dihydrotestosterone standard curve).
6. Add 5 µl chemical or standard/50 ml tube. This is a 1:1000 dilution of the chemical to the diluted yeast cells.
7. Incubate over night (~18 hours) at 30°C in shaking incubator at 300 rpm.
8. Following overnight incubation:

a. Make a 1:10 dilution of each tube in growth media and determine OD_{600}.

b. Dilute samples to OD_{600} of 0.25 in 1.5 ml microfuge tube.

c. Dispense 100 µl of diluted yeast/well of a 96 well plate. Do each dose of chemical or standard in triplicate.

d. Determine OD_{590} on microplate reader.

9. Set up plate reader to read blank and unknowns at 420 nm, for 20 minutes, with readings every minute.

10. Add 100 µl of Assay Buffer to each well.

For 11 ml of Assay Buffer:

2mg/ml ONGP	22 mg
0.1% SDS	110 µl 10% SDS
50 mM 2-Mercaptoethanol	29.7 µl 2-ME
200 U/ml oxalyticase	11 µl 200 U/µl oxalyticase
Z-Buffer	10.9 ml

- Make sure ONGP is in solution before adding SDS. Dilute ONGP in Z buffer in 50 ml polypropylene tube and vortex to mix.
- Stable for 1 hour. Use immediately after preparation.

11. Start reading immediately on microplate reader set at 420 nm every minute for 20 minutes. Samples will turn yellow as reaction occurs.
12. Determine Vmax (change in OD_{420}/minute) for the linear portion of the reaction.
13. Normalize the activity by calculating V_{max}/OD_{590}.