XTT Assay

Day 1:
Start 5 ml YPD culture
Set up template in notebook
Check to be sure reagents are available

Day 2:
Perform hemocytometer counts: cells/5 small boxes x 5 x 10^4
Dilute cells to 10^6 in RPMI (record bottle used in notes)
Place 100 µl in each well
-Include controls without cells
-Do not use outside wells, except for controls
Place 200 µl saline in outside wells
Parafilm and place in foil
Incubate 37^o for 24 hours

Day 3:
Prepare antifungal to have ready for after washes
Remove media
-If using same tips, must have same cell type in each well.
-Do not contaminate controls.
Wash gently with 100 µl PBS 2-3 x
-Be sure to remove all fluid each time
-New tip for each wash
-Place tip in corner and be careful not too disrupt the biofilm
Add 90 or 100 µl antifungal
-If making serial dilutions, must dilute in another plate to avoid transfer of cells.
-If making serial dilutions in another plate, transfer only 90 µl to biofilms.
Add 90 or 100 µl RPMI
-Add only 90 µl of RPMI if 90 µl of antifungal added
Incubate 24 hours at 37^o

Day 4
Thaw XTT/PMS or FDA
-Place in warm water if needed quickly
Centrifuge to remove sediment
Remove media
-Use new tip each well.
Wash gently with 100 µl PBS 2-3 x
-Be sure to remove all fluid each time
-New tip for each wash
-Place tip in corner and be careful not too disrupt the biofilm
Turn off direct lights
Add 90 µl XTT (0.5mg/ml or 1 mg/ml)
-Pipette each time, do not use multichannel
Add 10 µl PMS
-Okay to use multichannel, but only need to add about 6ml to Petri dish.
Place in foil and Incubate at 37^o 2 hours
Read at 490 nm

XTT (1mg/ml):
1mg/ml 1x PBS
Centrifuge to remove sediment
Collect supernatant
Store -20^{o} in foil

PMS (0.32 mg/ ml):
Add 9.6mg PMS to 30 ml sterile water
Place directly into foil and store -20^{o}