

Protocol :1 To quantify degradation of catechu (1%,0.5% & 0.1%) using Elisa plate reader

REQUIREMENTS :

glasswares /reagent /miscellaneous	Volume / Quantity
St.Eppendorf tubes	90
St.Suspension tubes	4
Micropipettes and st.tips	-
St. dilution tube	3
96 wells plates	5
St.M9+G+CS broth (1%,0.5% and 0.1%)	50 ml each
St.saline	10-15 ml

PROCEDURE :

1. Prepare st.M9+G+CS broth of 1% ,0.5% and 0.1% of catechu extract.
2. Prepare 5 sets of eppendorf containing 6 st.eppendorfs (4 tests + 2 control) each for each concentration (1%,0.5% and 0.1%),therefore total 18 st.eppendorf in one set for readings at different intervals.2 control includes media and media+kanamycin.
3. Dispense 1 ml of st.M9+G+CS broth of 1% ,0.5% and 0.1% (10%) in each set of tubes.
4. Prepare suspension of 1 OD of 4 isolates (S2C1 + S2C2 + XylE + CatA).
5. Inoculate 100 µl of culture suspension (10% inoculum) in eppendorf tubes (test)
6. Inoculate 100 µl of st.saline in eppendorf tubes (control) containing tannic acid solution
7. Before taking readings, centrifuge one set of eppendorf tube at 5000 rpm for 15 mins.
8. Dispense 200 µl of supernatant in 96 wells plate reader.
9. Take readings at 0hr and at different interval of time.

	1 %	0.5%	0.1%
S2C1			
S2C2			
CONTROL			
XylE			

