MCF-7 cell proliferation assay

1. MCF-7 cells (purchased , provided from cell line bank and stored in straws anchored in liquid nitrogen)

2. Phenol red-free D-media (EMEM with 50% increase of all essential amino acids except glutamine, 50% increase of all vitamins, and 100% increase of all non-essential amino acids) supplemented with 5% fetal bovine serum (FBS). Adjust pH , autoclave, filter and add PSN antibiotic mixture (3ml/l).

3. Cells are grown in phenol red-free D-media , incubated at 5% CO2, 95% air and 100% of humidity at was 37^{o}C.

4. Chemicals or drugs are diluted in the phenol red- free D media with 5% dextran-coated charcoal-stripped FBS (DCC-FBS) and 3ml/l PSN (test media) (Ethanol 0.1% in test media should be used as the vehicle; estradiol benzoat or other well known estrogenic compound are used as positive controls).

5. The cells ($5x10^4$/ml) plated in 6-well culture plate (2ml/well), in triplicate, are allowed to attach for 24h.

6. The phenol red-free D media was replaced with phenol red-free D media supplemented with 5% DCC-FBS (incubation for 24h)

7. Remove the media and replace with the test media containing test extracts followed by incubation (37^{o}C, 3 days, once change of the test media)

8. Harvest the cells by tripsin, dillute and count under microscope (with Newbauer's chamber).

And/or measurement of DNA isolated from the cultured cells can be alternative .