Appendix B1

Protocol for the Competitive ER binding MCF-7 (Whole Cell Assay)

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Stoessel and Leclercq, J Steroid Biochemistry **25**(5A):677-682, 1986.

PROTOCOL FOR THE COMPETITIVE ER BINDING

(Whole Cell Assay)

1. Culture conditions: MCF-7 cells are maintained at 37°C in a 5% CO_2 atmosphere in phenol red-free minimal essential medium (MEM) containing 10% charcoal stripped calf serum and penicillin, streptomycin, glutamine.

2. MCF-7 cells (20,000 cells/ml) are incubated for 4 days in 24 multiwells (NUNC) under above mentioned conditions.

3. After 4 days of culture, the medium is removed and the cells are incubated for 1 hour at 37° C with 1 nM [^{3}H]estradiol (E_2) or investigated compound (X) at concentrations ranging from 1 nM to 1µM.

4. Medium is again removed and the cells washed twice with phosphate buffer saline (PBS).

5. 250 µl absolute ethanol are added to each well (exposition during 20 min).

6. Aliquots of 200 µl of supernatant (ethanol extract) are added to 3.8 ml of scintillation liquid for radioactivity measurements (10 min, counting).

7. RBA data are established from the mean of 3 independent experiments, each performed in triplicate.

8. Relative binding affinity: $RBA = \dfrac{(I_{50})E_2}{(I_{50})\ x} \times 100$

   I_{50} = concentration producing 50% inhibition of [^{3}H]E_2 incorporation (dpm of ethanol extracts).

Brussels, December 2001.

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