Prostate specific antigen (PSA) production by cell lines other than those of prostatic origin has not, to our knowledge, been reported as yet. We have recently found that about 30% of breast tumors produce PSA. We here report the development of a system which reproduces this phenomenon in-vitro. The steroid hormone receptor-positive breast carcinoma cell line T-47D was cultured in microtiter plates and confluent cells were stimulated with a variety of steroids. After 48h we were able to detect PSA production, measured in the tissue culture supernatant with a highly sensitive immunofluorometric procedure (Clin Chem 1993;39:2108-14). The identity of the immunoreactive PSA species was established by high performance liquid chromatography and Western blot analysis. The most potent stimulating steroids were androgens (including testosterone and dihydroandrosterone), progestins (including norethynodrel and norgestrel) glycocorticosteroids (hydrocortisone) and mineralocortico-steroids (aldosterone). Estrogens (including β-estradiol and estrone) blocked the effects of androgens and progestins in competition experiments. These data are consistent with the hypothesis that PSA production in breast cancer is a steroid hormone receptor mediated event. Tamoxifen, a widely used breast cancer antitumor agent, was also able to induce PSA production in breast tumors using an in-vitro system. The in-vitro system may find applications in screening antitumor agents and for designing new therapeutic interventions based on drugs that target the steroid hormone receptors. Additionally, the new system may be used to study the mechanism of steroid hormone receptor-mediated gene expression.