

The following protocol for charcoal stripping fetal bovine serum is taken from "**STEROID HORMONES: A Practical Approach**" edited by B. Green and R.E. Leake, 1987, IRL Press, Oxford (pages 213-214).

"3.4 Preparation of charcoal-stripped and heat-inactivated fetal calf serum

Follow the procedure shown in *Table 9*. In our experience, this procedure will reduce the level of estrogen in the neat FCS to below 10^{-11} M, that is to below 10^{-12} M in the medium containing 10% FCS. Although this level is sufficiently low to permit most studies on estrogen-induced responses, it should not be assumed that such procedures entirely eliminate either the estrogen content or, indeed, the content of any other steroid family.

Remember that heat inactivation of serum also removes factors other than steroid. For example, in our studies of primary cultures, it was clear that, although the attachment of rat uterine cells to substrate was enhanced in FCS stripped with dextran-coated charcoal (DCC) at 4°C, attachment was reduced 3-fold after DCC stripping at 56°C. We have not characterized this attachment factor(s) but do find that it is consistently removed during heat inactivation of serum.

Product Information

Table 9. Preparation of charcoal-stripped serum.

1. Incubate overnight at 4°C Norit A charcoal [Sigma Prod. No. C3345] and dextran T-70 [Sigma Prod. No. D1390] in 0.25 M sucrose/1.5 mM $MgCl_2$ /10 mM Hepes pH 7.4 at final concentrations of 0.25% and 0.0025%, respectively.
2. Take a volume of the dextran-coated charcoal (DCC) equivalent to that of the serum which is to be stripped. Centrifuge it (500 g for 10 min) to pellet the charcoal.
3. Decant the supernatant and replace it with the same volume of FCS. Remember that each new batch of FCS, even from the same supplier, may have different growth characteristics from the last one and must be checked against some of your existing stock.
- 4*. Vortex the tube to thoroughly mix the charcoal with the serum and incubate either for 12 h at 4°C (DCC-stripped serum) or for 2 x 45 min at 56°C (heat-inactivated, DCC-stripped serum = HIDCC serum)."

* Heat - inactivation is also used to prepare complement-deficient serum.