Blocking Protocol

1) Transfect 293 cells or Cos cells or any easily transfactable cell line with SR-BI.

2) Next day, add DMEM with 0.2% BSA to the media plus 1:500 dilution (or 1:1000) dilution of the SR-BI blocking ab. Incubate for 30 minutes to 1 hour at 37 deg C.

3) Add 1 to 10ug/ml of radiolabeled or fluorescent HDL (labeled either on the lipid or protein) to cells for 1 to 2 hours (in the presence of the blocking antibody). For control cells, do not add blocking antibody.

4) Wash cells 3 to 4 times with ice cold PBS.

5) Measure HDL uptake by appropriate method (depending on label on HDL).

Note: As a positive control, you can add an excess (100-fold more) of unlabeled HDL to cells together with the label. This should block the uptake of labeled HDL by 80% or more. This positive control should tell you that your cells are expressing functional SR-BI. Also, cells not receiving either unlabeled HDL or no blocking ab should tell you that your cells are expressing functional SR-BI.