Transfection of endothelial cells essay sample

Transfection for HMECs using Lipofectamine LTX + Plus reagent NB: • Using PLUS™ Reagent (Cat. No. 11514-015) enhances transfection performance in HUVEC cells. • The addition of antibiotics to media during transfection may result in cell death

Transfection of HMECs

Use this procedure to transfect plasmid DNA into HMECs cells in a 12-well format

All amounts and volumes are given on a per well basis.

1. Cell density should be 50~80% confluent on the day of transfection (use the normal growth medium without antibiotics). 2. For each well of cells to be transfected, dilute 1 µg of DNA into 200 µl of Opti-MEM® Medium without serum. 3. Mix PLUS™ Reagent gently before use, then add 1 µl PLUS™ Reagent (a 1: 1 ratio to DNA) directly to the diluted DNA. Mix gently and incubate for 5-15 minutes at room temperature. 4. For each well of cells, dilute 4 µl of Lipofectamine™ LTX into the above diluted DNA solution, mix gently and incubate for 25 minutes at room temperature to form DNA-Lipofectamine™ LTX complexes. 5. Remove growth medium from cells and replace with 1 ml of complete growth medium without antibiotics. Add 200 µl of the DNA-Lipofectamine™ LTX complexes directly to each well containing cells and mix gently by rocking the plate back and forth. 6. Complexes do not have to be removed following transfection. Incubate the cells at 37°C in a CO 2 incubator for 18-24 hours post-transfection before assaying for transgene expression.