The AfCS is utilizing antisense technology to manipulate signaling protein expression in the RAW 264.7 macrophage-like cell line. This can be achieved by the transfection of gene-specific antisense oligonucleotides (ASOs), acquired from Isis Pharmaceuticals (http://www.isispharm.com/). The following procedure involves the transfection of ASOs into RAW 264.7 cells using FuGENE 6 transfection reagent. Subsequently, the isolated total RNA or protein from these transfected cells can be used to assess the level of mRNA or protein knockdown, respectively.

**Day 1**
1. Count RAW 264.7 cells and plate in a 24-well plate at $1 \times 10^5$ cells/well, 1 ml/well.
2. Incubate cells at 37 °C in a humidified, 5% CO₂ incubator for 24 hr.

**Day 2**
1. Add 78 µl Opti-MEM to a 15-ml polypropylene tube.
2. Add 2 µl FuGENE 6 directly to medium and mix gently. Do not allow FuGENE 6 to come into direct contact with plastic surface of tube.
3. Add 20 µl of 10 µM stock ASO.
4. Incubate at room temperature for 30 min.
5. During the 30 min incubation, aspirate medium from cells and add 400 µl of fresh RAW 264.7 growth medium 1 (RAWGM1) to cells.
6. Add FuGENE 6 complexes to wells (100 µl/well). Mix gently by rocking the plate, and incubate cells for 24 hr at 37 °C in a humidified, 5% CO₂ incubator.

**Day 3**
1. Aspirate medium and add 1 ml fresh medium per well.
2. Continue to incubate cells at 37 °C in a humidified, 5% CO₂ incubator.

**Day 4**
1. Harvest cells for RNA analysis as indicated in Cell Lysis and Total RNA Isolation from RAW 264.7 Cells, AfCS protocol PP00000260.
2. For cells destined for protein analysis, change medium on cells.
3. Aspirate medium and add 1ml fresh medium per well.

**Day 5**
1. Harvest cells for protein analysis as indicated in Cell Lysis and Protein Extraction from RAW 264.7 Cells, AfCS protocol PP00000258.
Reagents and Materials
Cell culture cluster, 24 well: Corning Inc.; catalog no. 3526

Opti-MEM I reduced-serum medium (1X) liquid: Invitrogen; catalog no. 31985088

Polypropylene tube, 15 ml: VWR; catalog no. 20171-012

FuGENE 6 transfection reagent: Roche; catalog no. 1814443

RAW 264.7 growth medium 1 (RAWGM1): AfCS Protocol ID PS00000510

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