Procedures for MesoScale Discovery (MSD) assay development—MYOstatin

Timing-1hr block—1 hr BT-coating Ab—2 hrs sample adsorption—1 hr Ru-reporter Ab binding—read buffer T immediately; total = 5 hrs incubation time ++++ 4 washes ++reagent transfers

Exceptions may be noted in individual experimental protocols

Wash MSD Streptavidin Gold plates w/TBST (LEM MSD 3X wash program)

Block MSD Streptavidin Gold plates (cat# L15SA-1/L11SA-1) with 250 ul/well of TBS+1% OVALBUMIN(Sigma cat#A5378 lot#SLBB43410V) for **1 hr** @ RT w/mixing (make 30 ml/plate)

Wash plate 3X with TBST (LEM MSD 3X wash program)

Coat plate with 50 ul/well of biotin-labelled primary capture Ab **BT-IBA139** @ 0.5 ug/ml in TBST/0.1% OVAL for **1 hr** @ RT w/mixing (make about 7.5 ml/plate)

Wash plate 3X with TBST (LEM MSD 3X wash program)

add 50 ul/well of **samples**-pre-diluted (MRD=4) in polypropylene plate-w/LEM assay buffer +1% OVALBUMIN+0.1 mg/ml Scantibody (HBR1 MMDDYY lot### @ 0.1 mg/ml) for **2 hrs** @ RT w/mixing

include reference standard curve (MRD=1; in duplicate) on each plate for calibration purposes; for **Myostatin** (Reagent Proteins catalog#CYT-418; make [stock] @ 10 ug/ml in LEM assay buffer + 1% OVALBUMIN+0.1 mg/ml Scantibody); use @ 1000 ng/ml dilute down 3X steps

Wash plate 3X w/TBST (LEM MSD 3X wash program)

add 50 ul/well of ruthenium-labelled secondary reporter Ab **Ru-IBA140** @ 0.1 ug/ml in LEM assay buffer+0.1% OVAL +0.1 mg/ml Scantibody for **1 hr** @ RT w/mixing (make about 7.5 ml/plate)

Wash plate 3X with TBST (LEM MSD 3X wash program)

add 150 ul/well of **MSD Read Buffer T** w/surfactant diluted 1:1 with H_2O from 4X stock to 2X (make 20 ml/plate); mix briefly

Read plate(s) in MSD SECTOR instrument **ASAP,** *ie within minutes*