

Public Abstract

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Title:Development of a Fibroblast Growth Factor 21 Assay

Obesity and diabetes play a major role in many diseases prevalent in our current society. Fibroblast Growth Factor 21 (FGF21) is a recently discovered hormone that has potential for therapeutic treatment of obesity and diabetes. It acts as a hormone in times of metabolic stress and is shown to stimulate hepatic fatty acid oxidation, ketogenesis, induce weight loss, allow for sustained decrease in plasma glucose and triglycerides, and decrease the growth hormone response. Obese or diabetic individuals often have higher than normal amounts of circulating FGF21; consequently, they may be considered FGF21 resistant. It is then advantageous to have an assay to measure FGF21 that will allow us to study FGF21 further. It is also important to study FGF21 in various livestock species for potential biomarkers, or as indicators of carcass quality that could prove to advance further research. We have developed a working FGF21 assay using an amplified luminescent proximity homogeneous assay (AlphaLISA). It is a homogeneous, no-wash immunoassay with high sensitivity and wide dynamic range. It is a bead based technology that brings two antibodies near each other allowing for an unstable singlet oxygen to trigger a downstream cascade of chemical events leading to a sharp intense chemiluminescent emission that can be read on an EnVision machine. The assay was set up using a 'sandwich' design with guinea pig or rabbit anti-bovine FGF21 antibodies attached to the acceptor beads and biotinylated rabbit anti-bovine FGF21 antibodies attached to a streptavidin coated donor bead. The assay has a working range from 0.2-200 ng/ml. Standard curves in serum from bovine, porcine, rabbit, rat, ewe, fetal bovine has been conducted and assay conditions were optimized for each. Manipulations of the sera were necessary in order to get functional curves. These included diluting it 2 fold or more, depending on the species, and increasing incubation temperatures. The assay can function using both serum and plasma.