RELATIVE POTENCY ASSAY FOR VIRAL VECTOR ENCODING ISOMEROHYDROLASES

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/328,916, filed April 28, 2016. The entire contents of the foregoing application is incorporated herein by reference, including all text, tables, sequence listing and drawings.

INTRODUCTION

[0002] Isomerohydrolase activity or potency can be measured by quantifying reaction products produced by such enzymes. The invention relates to measuring isomerohydrolase activity and/or potency.

SUMMARY

[0003] The invention provides methods for measuring and/or detecting isomerohydrolase activity. In certain embodiments, methods include the use of a non-radioactive isomerohydrolase substrate or precursor of the isomerohydrolase substrate and detection of the non-radioactive reaction product produced by conversion by the isomerohydrolase. In certain embodiments, methods include the use of mass spectrometry to quantify the reaction product thereby measuring and/or detecting isomerohydrolase activity.

[0004] In a particular embodiment, a method for measuring and/or detecting isomerohydrolase activity includes (a) contacting cells (e.g., eukaryotic cells) expressing Lecithin Retinol Acyltransferase (LRAT) with a viral vector (e.g., an adeno-associated viral (AAV) vector) comprising a transgene encoding an isomerohydrolase protein (e.g., RPE65) under conditions allowing cell transduction; incubating viral vector transduced cells under conditions allowing expression of the encoded isomerohydrolase protein (e.g., RPE65); lysing the transduced cells to produce an extract (e.g., cell extract) comprising the encoded isomerohydrolase protein; incubating the extract (e.g., cell extract) with a substrate for a period of time and under conditions allowing conversion of the substrate by the isomerohydrolase protein to a reaction product; subjecting the reaction product to column (liquid) chromatography thereby producing a column (liquid) chromatography purified reaction product; and subjecting said column (liquid) chromatography purified reaction product to mass spectrometry thereby quantifying the reaction product, wherein the amount of reaction product reflects isomerohydrolase activity thereby measuring isomerohydrolase activity.