www.crownbio.com
3375 Scott Blvd., Suite 108, Santa Clara, CA 95054
Contact: Tel. 855.827.6968 / Fax. 408.884.2339
products@crownbio.com

Protocol

# Wnt Reporter Assay with 293T Wnt Luciferase Cell Line

## Materials & instruments

- Cell line: 293T Wnt luciferase cell line (Crown Bioscience, catalog # C2006)
- Wnt: Commercial or conditional medium from L-wnt3a cell line (ATCC, catalog # CRL-2647). To make conditional medium from L-wnt3a, seed cells at 80-90% confluence followed by collecting supernatant 72-96 hours later. Centrifuge, filter, aliquot and store at -80 for future use. Test its activity before formal study.
- Wnt3a: recombinant protein, homemade or commercial (R&D Systems 1324-WN)
- R-spondin: recombinant protein, homemade or commercial (R&D systems, catalog # 3266-RS and/or 3500-RS)
- One-Glo™ luciferase assay kit: Promega, catalog # E6110
- Plate: Corning, catalog # 3610 or others suitable for luciferase detection
- Plate reader: Envision 2104 Multilabel Reader (Perkin Elmer) or equivalent

## Simplified workflow with Wnt/R-spondin

Seed cells at 20,000/well in 96-well plate

⇓ Culture for at least 6 hours in 37°C, 5% $CO_2$ incubator

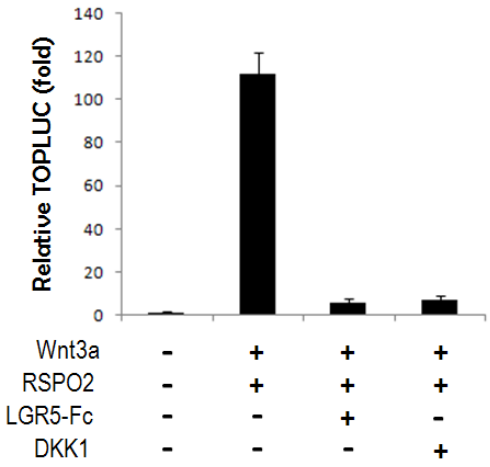
Add appropriate Wnt, R-spondin

⇓ Culture for 20-24 hours in 37°C, 5% $CO_2$ incubator

Add firefly luciferase substrate according to user manual

⇓

Detect on plate reader




**Example figure**
293T Wnt luciferase cells seeded in 96-well plate were stimulated with soluble Wnt3a and R-spondin 2 in the absence and presence of Wnt inhibitory protein LGR5-Fc or DKK1 for 20 hours, followed by luciferase activity measurement.