

# Validation Report #029816

Validation Date: 09/18/14

## Summary

Antigen	Beta Catenin
Catalog number	<a href="#">ABIN1043907</a>
Supplier	antibodies-online
Lot number	27397
Method validated	<a href="#">Western Blot</a>
Laboratory	<a href="#">ADS Biosystems Inc</a>
Validation number	<a href="#">029816</a>
Positive Control	<a href="#">MCF-7 cells</a>
Negative Control	<a href="#">SK-BR-3 cells</a>
Notes	A strong specific band was observed in the positive control at the expected size (~85.5 kDa) that is not observed in the negative control.



# Full Methods

## **Primary Antibody**

- Antigen: Beta Catenin
- Catalog number: ABIN1043907
- Supplier: antibodies-online
- Lot number: 27397
- Dilution: 1:1,000

## **Loading Control Antibody**

- Antigen: Alpha-tubulin
- Supplier: Life Technologies
- Supplier catalog number: 322500
- Lot number: 1439416A
- Dilution: 1:10,000

## **Secondary Antibody**

- Antibody: IRDye 680LT Goat Anti-Rabbit
- Catalogue number: 827-11081
- Supplier: LI-COR Biosciences
- Lot number: C30725-01
- Dilution: 1:10,000

## **Additional Information**

### **Controls**

- MCF-7 lysates were prepared by ADS Biosystems following standard protocols and quality controlled for protein integrity on a regular basis
- SK-BR-3 lysates were purchased from Santa Cruz Biotechnology (catalog number sc-2218)

### **Protocol**

- Lysates were mixed with NuPAGE® LDS Sample Buffer (Life Technologies NP0007) and NuPAGE® Sample Reducing Agent (Life Technologies NP0004) and denatured for 5 minutes at 90°C.
- 40 µg of each lysate was electrophoresed on a Bolt 4-12% Bis-Tris Gel (Life Technologies BG04120BOX) and run in Bolt MOPS SDS Running Buffer (Life Technologies B0001) at 160 volts for 1 hour.
- Odyssey Western Protein Standard (LI-COR #928-40000) was run as a molecular weight standard.
- PVDF membrane was activated with methanol.
- Protein samples were transferred to activated PVDF membrane in a wet Bolt Transfer Apparatus (Life Technologies B1000) at room temperature for 1 hour at 20 volts (started at 230mA, ended at 110mA).
- The membrane was blocked in x LI-COR Odyssey WB block solution for 1 hour at room temperature.
- The membrane was incubated with the primary antibody diluted 1:1000 in x LI-COR Odyssey WB block solution incubated 2 hours at room temperature.
- The membrane was washed 4 x 5 minutes in 1 x PBS-T (PBS solution with 0.1% Tween 20).
- The membrane was incubated with IRDye® 800CW Goat anti-Mouse Secondary Antibody (Red) and IRDye 680LT Goat Anti-Rabbit Secondary Antibody (Green) from LI-COR (#827-11081, Lot #C30725-01), both 1:10,000 dilutions. Incubation was performed at room temperature for 45 minutes.
- The membrane was washed 4 x 5 minutes in 1 x PBS-T (PBS solution with 0.1% Tween 20).
- Proteins were detected using Odyssey machine scanning with green channel for loading control and red channel for potential LPL band.

### **Experimental Notes**

- No experimental challenges noted.

## Figures

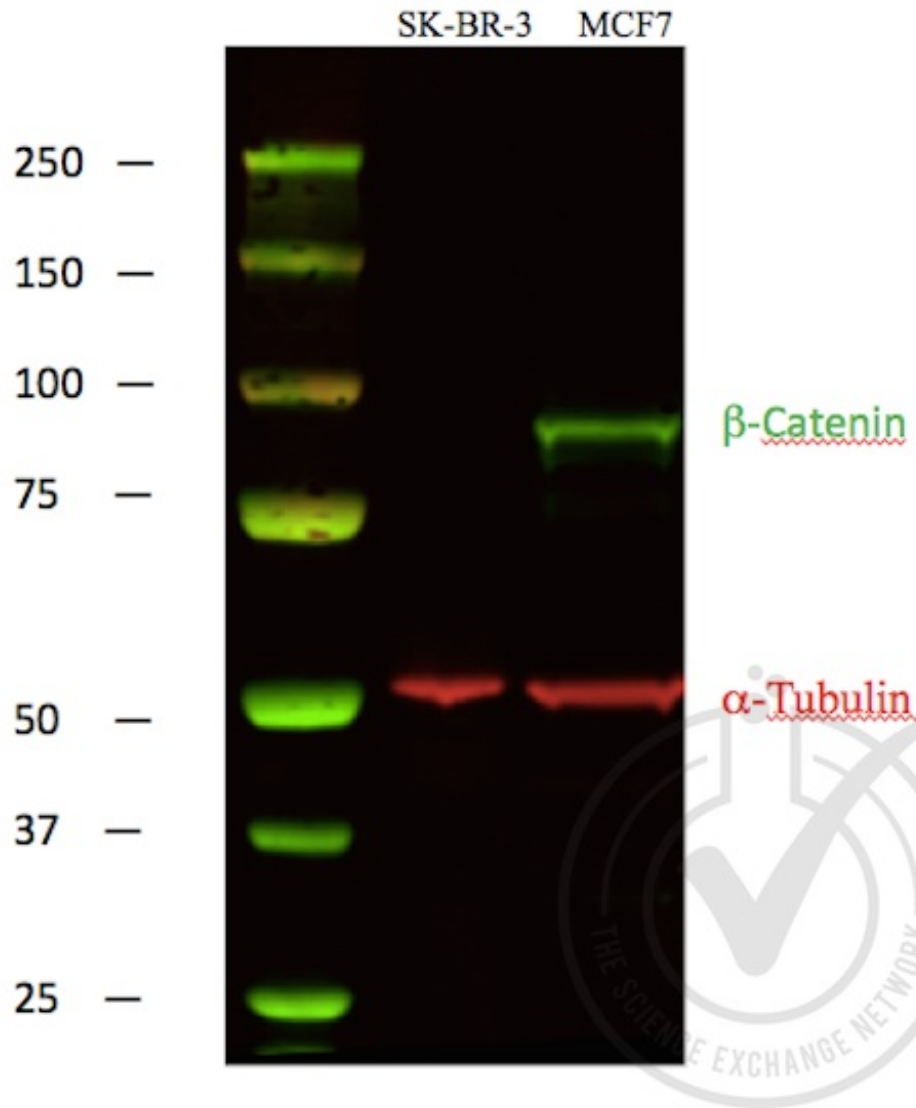


Figure 1: Scanned image of Beta Catenin (Green) and loading control alpha-tubulin (Red) Western blot using LI-COR Odyssey Infrared Technology. First lane, protein molecular weight markers. Second lane, SK-BR-3 negative control lysate. Third lane, MCF-7 positive control lysate.