

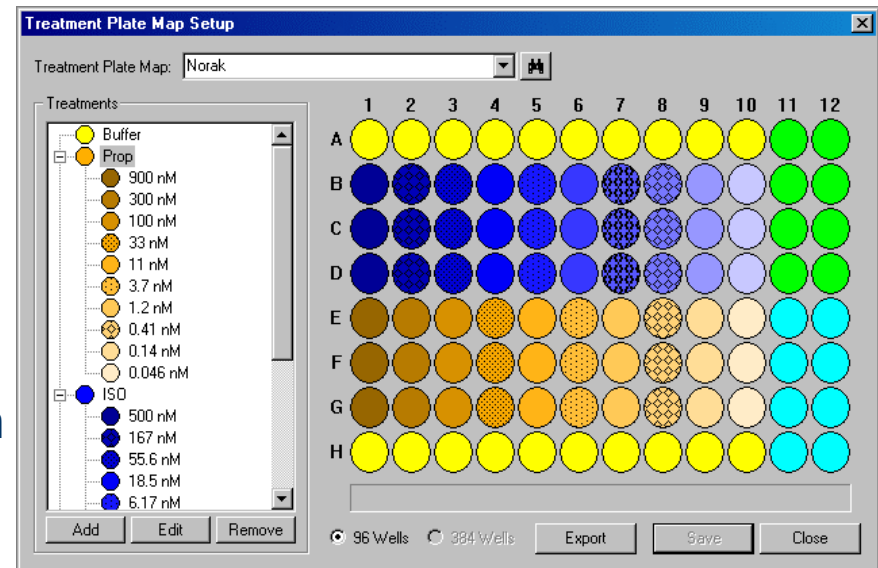
# Norak's Transfluor® GPCR Assay on Atto Bioscience's Pathway HT

**Pathway HT™**  
**Automated Bioimaging System**



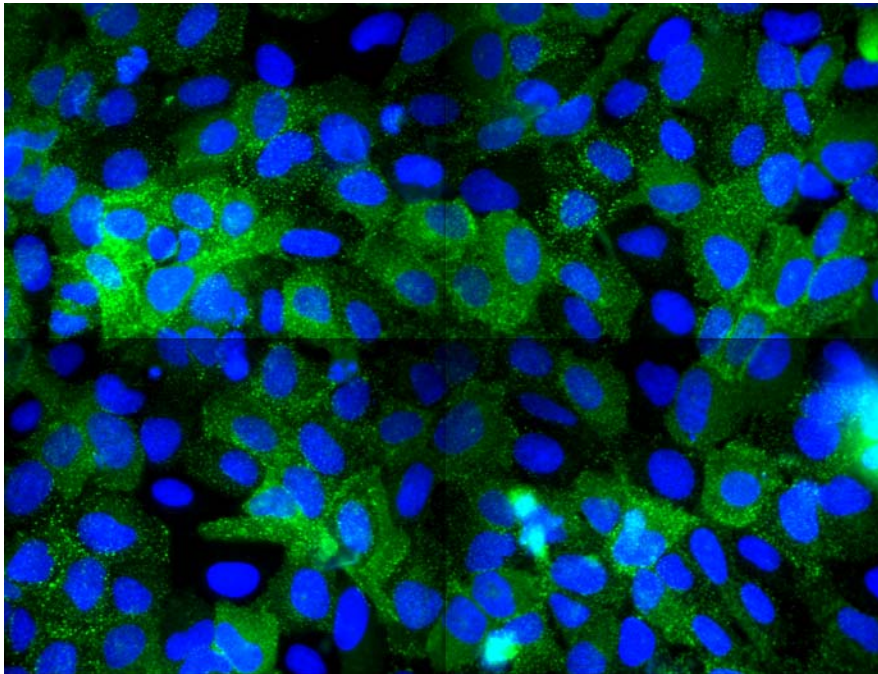
## Experiment Details

- Plate #1: Dose response
  - Wild type beta2 adrenergic receptor (“pit” forming)
  - Agonist: Isoproterenol (500nM-0.0254nM)
  - Antagonist: Propranolol (900nM-0.046nM)
- Plate #2: Dose response
  - Modified beta2 adrenergic receptor (“vesicle” forming)
  - Agonist: Isoproterenol (500nM-0.0254nM)
  - Antagonist: Propranolol (900nM-0.046nM)
- Automated Imaging Setting:
  - 40X Olympus objective
  - 4 Images per well montage
  - Dapi and FITC filter sets
  - Intensity granularity was measured
  - Measurements were made in the cytoplasm

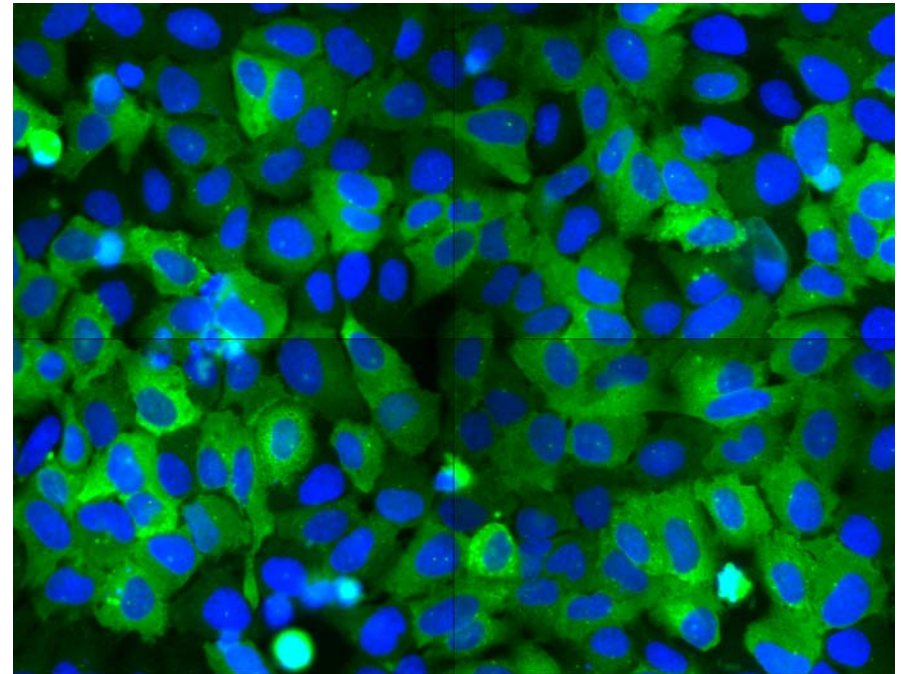


# Modified Beta2 Adrenergic Receptor

**Vesicle Forming Translocation**

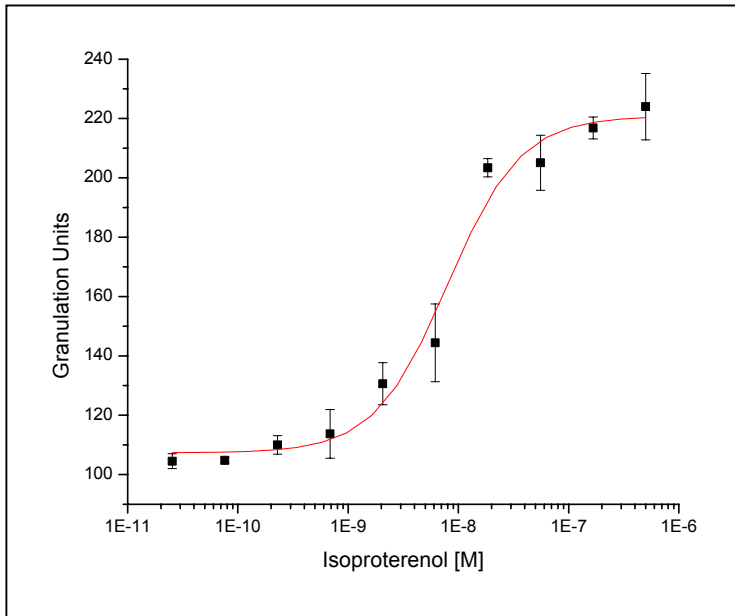


**Control**



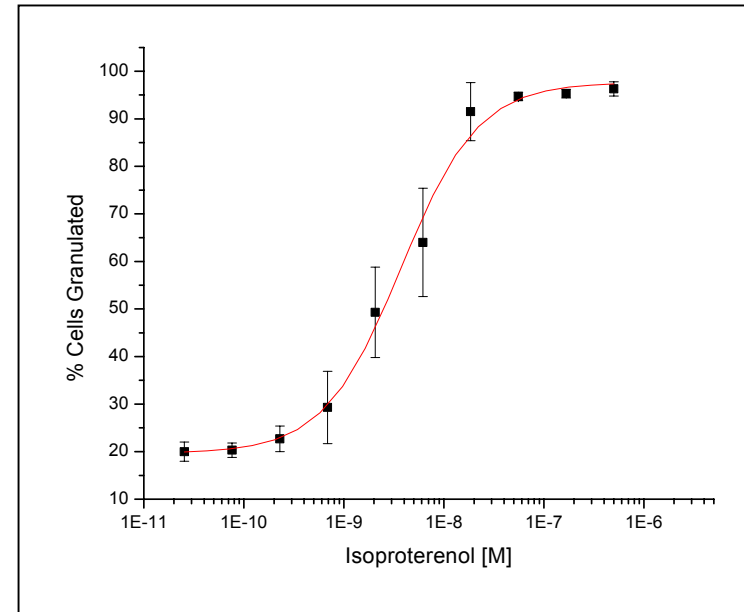
# Modified Beta2 Adrenergic Receptor - Isoproterenol

## Granulated Units



Parameter	Value	Error
Chi <sup>2</sup>	60.74758	
Initial(A1)	107.31723	4.43139
Final (A2)	220.80082	5.95394
EC50 (x0)	7.99E-09	1.64E-09
Power (p)	1.30997	0.31504

## % Cells Granulated

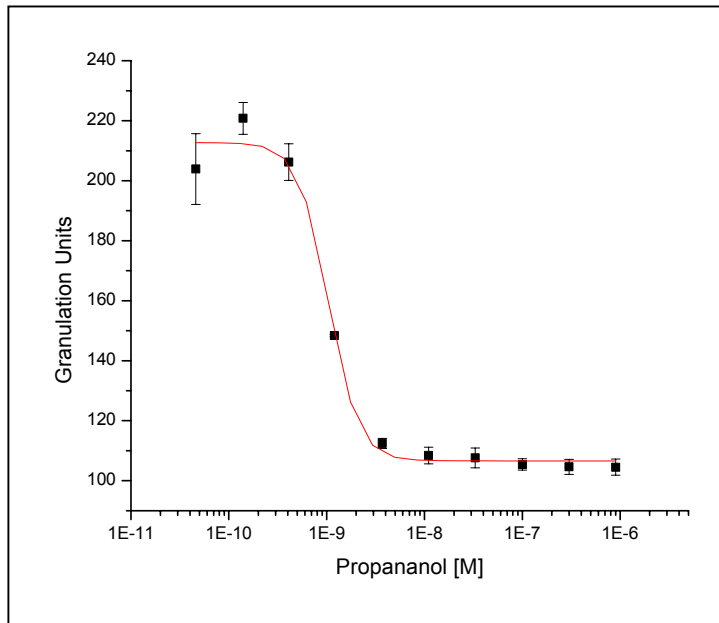


Parameter	Value	Error
Chi <sup>2</sup>	11.16169	
Initial(A1)	19.66131	2.34823
Final (A2)	97.6584	2.39046
EC50 (x0)	3.73E-09	5.37E-10
Power (p)	1.12562	0.16762



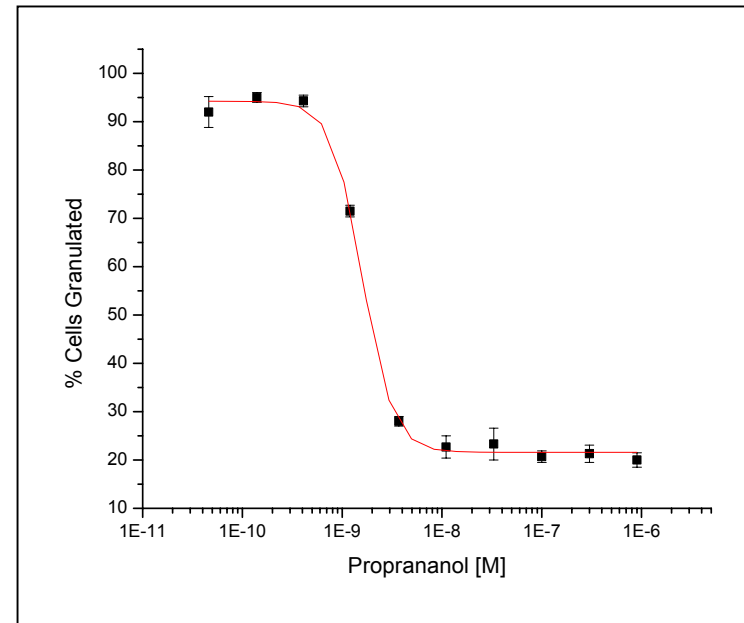
# Modified Beta2 Adrenergic Receptor - Propranolol

## Granulated Units



Parameter	Value	Error
Chi^2	2.54756	
Initial(A1)	94.22831	0.9803
Final (A2)	21.58373	0.72664
IC50 (x0)	1.59E-09	6.63E-11
Power (p)	2.83764	0.28497

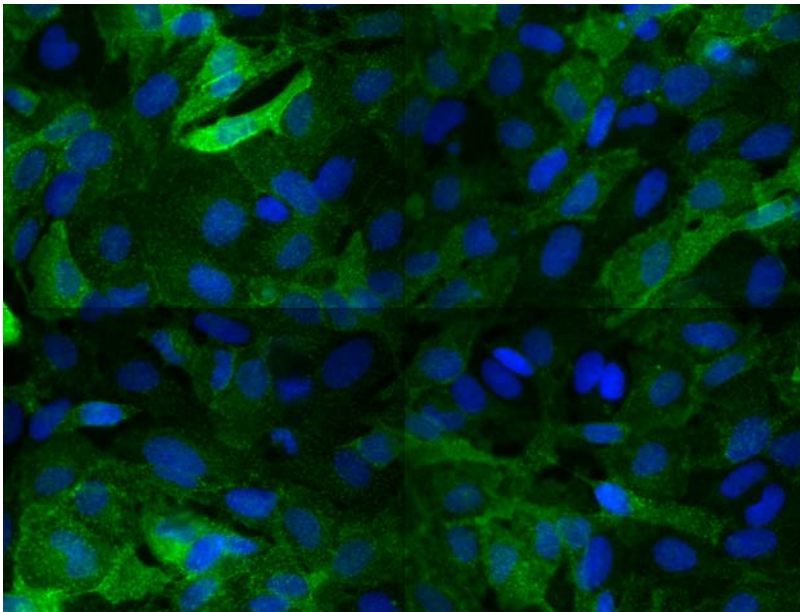
## % Cells Granulated



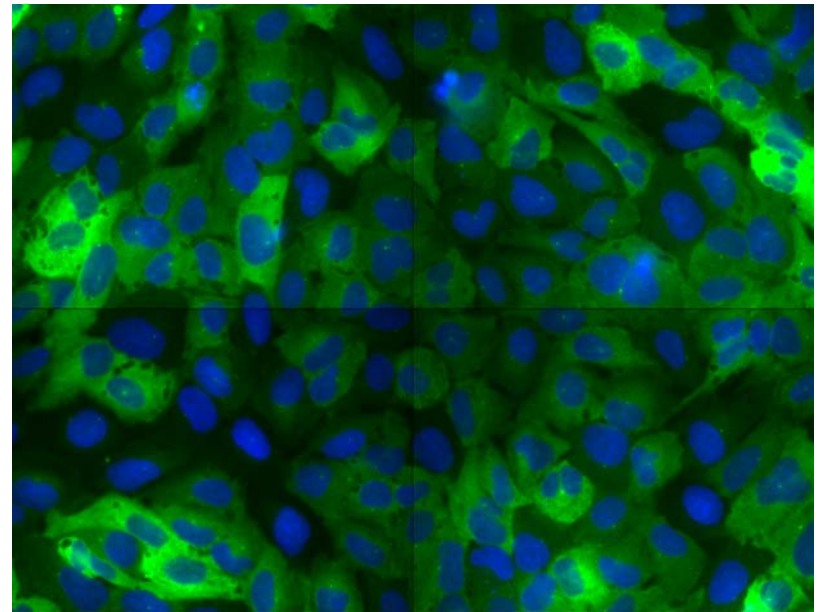
Parameter	Value	Error
Chi^2	28.65143	
Initial(A1)	212.6932	3.88463
Final (A2)	106.6008	2.27942
IC50 (x0)	1.04E-09	9.19E-11
Power (p)	2.84411	0.84787

# Wild Type Beta2 Adrenergic Receptor

**Pit Forming Translocation**

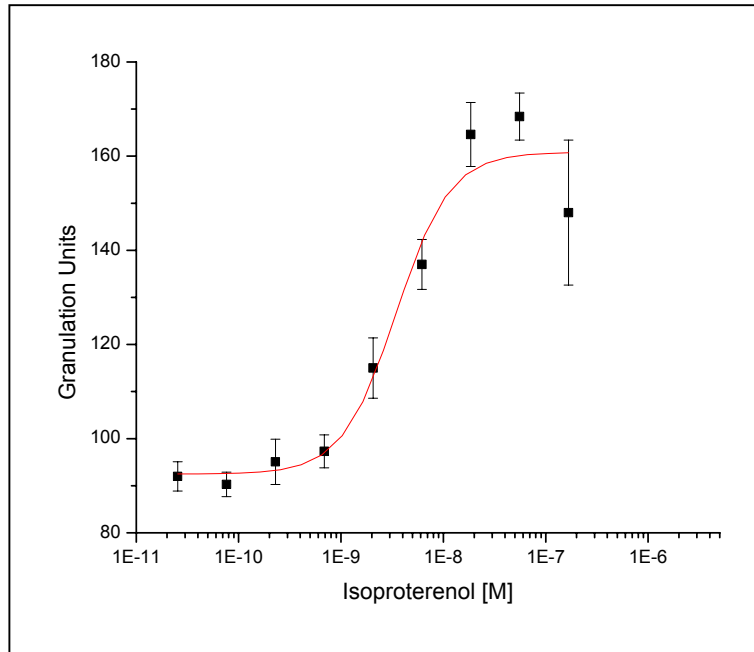


**Control**



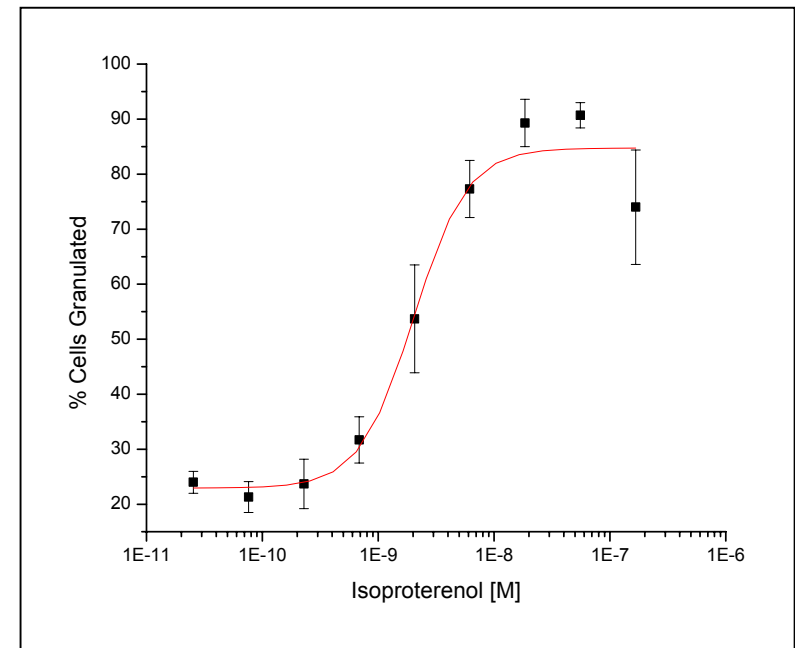
# Wild Type Beta2 Adrenergic Receptor - Isoproterenol

## Granulated Units



Parameter	Value	Error
Chi^2	65.61387	
Initial(A1)	92.50006	4.74259
Final (A2)	160.8152	5.73587
EC50 (x0)	3.46E-09	1.04E-09
Power (p)	1.65306	0.70364

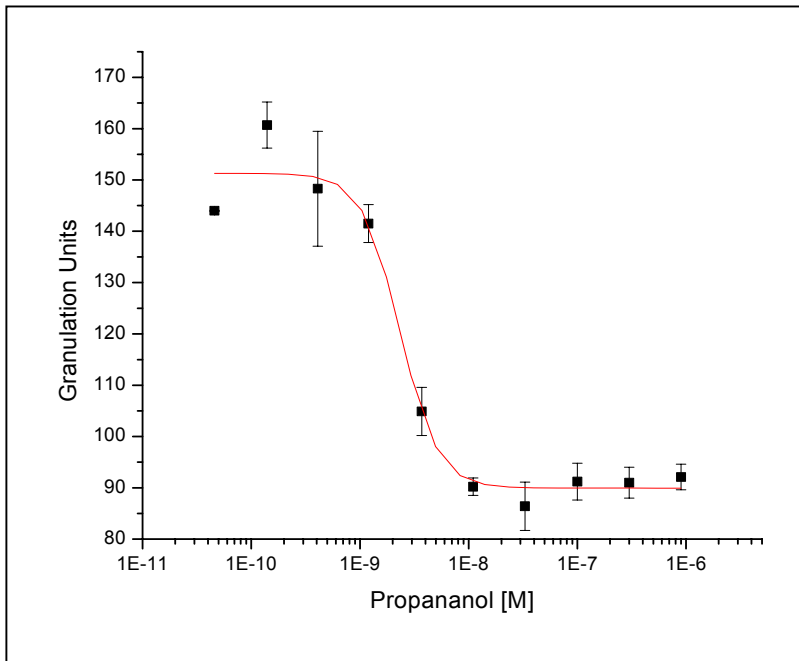
## % Cells Granulated



Parameter	Value	Error
Chi^2	38.01861	
Initial(A1)	22.93425	3.80985
Final (A2)	84.75487	3.7771
EC50 (x0)	2.02E-09	4.52E-10
Power (p)	1.86692	0.7449

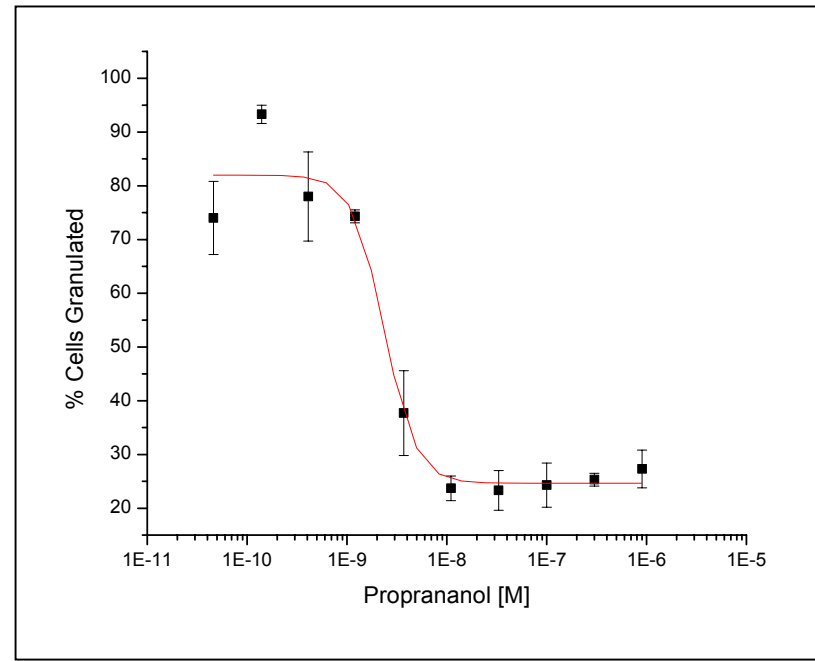
# Wild Type Beta2 Adrenergic Receptor - Propranolol

## Granulated Units



Parameter	Value	Error
Chi^2	28.17212	
Initial(A1)	151.3083	3.25304
Final (A2)	89.94803	2.47203
IC50 (x0)	2.33E-09	4.17E-10
Power (p)	2.49446	0.76258

## % Cells Granulated



Parameter	Value	Error
Chi^2	36.22991	
Initial(A1)	81.98675	3.62838
Final (A2)	24.64058	2.77269
IC50 (x0)	2.35E-09	5.04E-10
Power (p)	2.74038	1.04025



# EC50/IC50 Data Summary

Method	Modified	Modified	Wild Type	Wild Type
	Isoproterenol	Propranolol	Isoproterenol	Propranolol
	<u>EC50</u>	<u>IC50</u>	<u>EC50</u>	<u>IC50</u>
Norak Data	3.12E-09	1.32E-09	4.51E-09	8.69E-10
Granulated Units	7.99E-09	1.04E-09	3.46E-09	2.33E-09
% Cells Granulated	3.73E-09	1.59E-09	2.02E-09	2.35E-09

## Data Summary Notes

- The results for Wild Type and Modified Beta2 Adrenergic receptors for both the agonist (Isoproterenol) and antagonist (Propranolol) were similar to those provide by Norak. (Generated using the InCell3000 system)
- The presence of vesicles or pits were measured using Atto's granularity algorithm which calculates the variation of fluorescent intensity (degree of variation) within a region of interest. The variation is represented as Granulated Units.
- The data was presented in two ways: Granulated Units and % Cell Granulated. % Cells Granulated was calculated by measuring the % of the cells with Granulated Units above a threshold.
- The data points for the 900nM Isoproterenol concentration were not included in the Wild Type Beta2 graph because they were outliers. These wells were used during assay development and were repeatedly imaged, therefore some photobleaching did occur and which influenced the data.