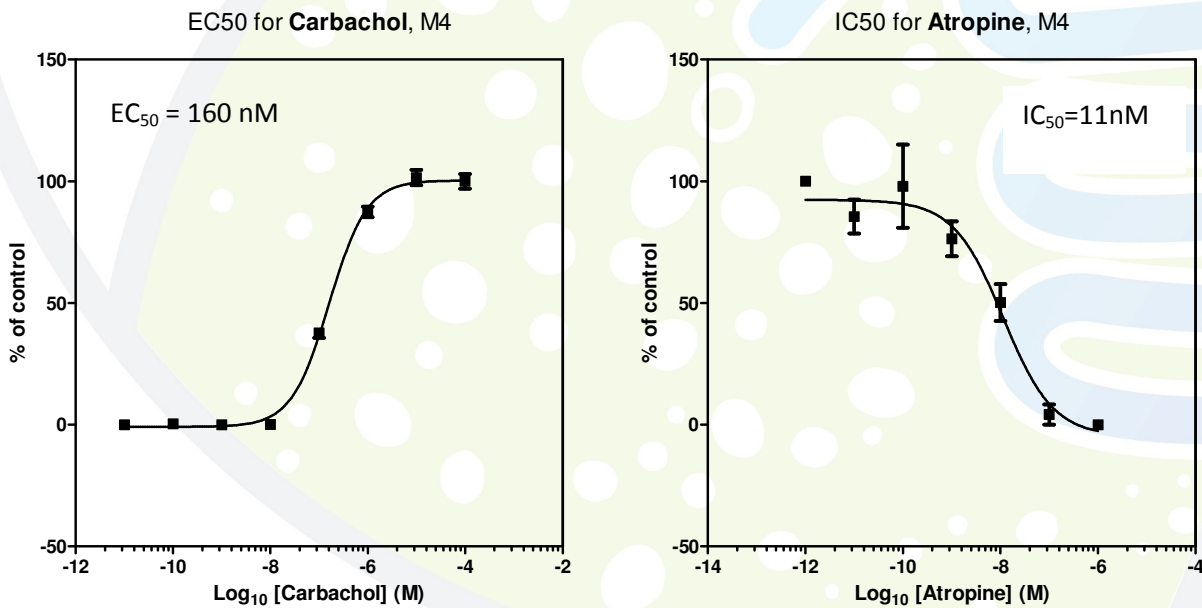


## GPCR Profiling

Target:	Muscarinic M4
Available Assays:	Radioligand Binding & Cell-Based
Cell-Based Agonist:	Carbachol
Cell-Based Antagonist:	Atropine



GPCR-expressing cells were plated in black, clear-bottomed 96 well plates at a density of 25,000 cells per well in 100µl volume and incubated overnight. Next day, medium was removed and 25 µl HBSS buffer (HEPES buffered Hank's balanced salt solution) was added to the wells. 10 µl calcium 5 dye (prepared according to the manufacturer's instructions in HBSS) was added and plates incubated at 37°C for 1 hour. If appropriate, antagonists were added and incubated for 5 min at RT. The plates were then placed in a fluorescence reader whereupon agonist was added and fluorescence monitored.