



## **Cell Binding/Internalization**

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

*Some of the NCL's experiments are tailored specifically to the individual nanoparticle under study. As such, a standard experimental method cannot be generated. Instead of a protocol, we offer the following brief description of the experiment and the types of data it generates, to aid in determining a suitable experimental pathway for a nanoparticle characterization project. The following description is **not** a protocol and does not describe all of the relevant experimental parameters necessary to conduct the experiment. If you'd like more information, please feel free to contact us at the phone number or email address provided on the previous page.*

## Cell Binding/Internalization

NCL has methods to assess targeting by measuring the inhibition coefficient ( $K_i$ ) and binding coefficient ( $K_d$ ) of a targeted nanoparticle.

### Inhibition coefficient ( $K_i$ )

To confirm  $K_i$ , we use an antagonism model that relates free target ligand concentration and targeted nanoparticle concentration-growth inhibition curves. The model has three parameters: maximal growth inhibition ( $E_{max}$ ), concentration at 50% growth inhibition ( $EC_{50}$ ), and free target ligand inhibition constant ( $K_i$ ). The parameters are related by a sigmoidal Michaelis-Menten antagonism equation:

$$E = \frac{E_{max}}{1 + 10^{((\log EC_{50} (1 + \frac{1}{K_i})) - X)\gamma}}$$

This equation assumes competitive single-site antagonism. The nanoparticle concentrations are expressed on a log scale as there are advantages to using a log scale for estimating the confidence intervals for  $EC_{50}$ . The log scale results in a sigmoidal concentration response-curve, hence the use of a sigmoidal model equation. The sigmoidal equation has the extra Hill coefficient,  $\gamma$ , a measure of sigmoidicity. The model parameters,  $E_{max}$ ,  $EC_{50}$ ,  $K_i$  and  $\gamma$ , are fit to all concentration-response data simultaneously. The values  $E_{max}$  and  $EC_{50}$  are measures of potency, while  $K_i$  is a measure of target ligand receptor interaction. Comparison of these estimated parameters between new lots and standard reference material, may help identify changes in target ligand density, drug loading and drug release properties.

### Binding coefficient ( $K_d$ )

To confirm the apparent  $K_d$  of the ligand and the  $B_{max}$  (maximum number of binding sites), the binding curves will be determined in a dose-response ligand-drug concentration range. The cells expressing the receptor are incubated with targeted nanoparticles in the absence and in the presence of free ligand (to determine total binding and nonspecific binding, respectively).

In the absence of free ligand, total binding (both specific and non-specific) is observed. In the presence of large amounts of free ligand, only nonspecific binding is observed. The total – nonspecific = specific binding, which should be a hyperbolic curve, with an asymptote approaching  $B_{max}$ . The difference in the values for total binding and nonspecific binding will yield the specific binding constant of the ligand ( $K_d$ ) for a one-site binding model:

$$B = \frac{B_{max} X}{K_d + X}$$

Where  $X$  is the concentration of the ligand-bound drug.