Validation of Na⁺, K⁺-ATPase Isoform Endogenous to Cardiomyocytes for High Throughput Rb Uptake Assay using Cor. At® Cardiomyocytes & ICR8000™

Introduction

Human Na⁺, K⁺ ATPase is the target for cardioglycosides such as digitoxin and digoxin which are used in the treatment of congestive heart failure and related conditions; thus, it is emerging as an important drug target. The Na⁺, K⁺ pump generates electrochemical gradients that are used to drive the coupled transport of many ions and nutrients across the plasma membrane as it actively exports three Na⁺ ions with the concomitant import of two K⁺ ions hydrolyzing one ATP molecule in the process.

In non-cell-based assays, the activity of Na⁺, K⁺ ATPase has been determined by using purified enzyme preparations to hydrolyze ATP. In cell-based assays, the techniques such as patch clamping, fluorescence, H⁺-Ouabain binding, and radio-tracer (Rubidium), and cold Rubidium flux assays have been used in either recombinant cell lines or in cells other than primary cardiomyocytes. However, the primary cardiomyocytes cannot be used, as they lack homogeneity, sensitivity and surface binding properties, in developing cell based assays in a HTS format.

In view of the availability of standardized pure cardiac myocytes with functional expression of all essential cardiac ion channels, Aurora Biomed validated Na⁺, K⁺ ATPase in cultured Cor.At® cardiomyocytes derived from transgenic mouse embryonic stem cells.

Materials & Methods

Rb⁺ uptake by cultured Cor.At® cardiomyocytes was carried as follows:

1. Culture and maintenance of Cor.At® cardiomyocytes: The cells endogenously expressing Na⁺, K⁺ ATPase were provided frozen in 96 well MTPs, thawed and cultured as per the instructions supplied by the supplier of the cells. On the 4th day of the culture, Rb⁺ uptake experiment was carried out.

2. Rb⁺ Uptake: Cells were washed once with 200 µl of Rb⁺ Uptake Buffer and Rb⁺ uptake was started by applying 200 µl Rb⁺ Uptake Buffer.
   a. The Rb⁺ uptake profile: The Rb⁺ uptake activity of the cells was studied by incubating the cells at specific time intervals in the presence of Aurora Biomed’s Rb⁺ uptake buffer.
   b. Dose response: The cells were incubated in the Rb⁺ uptake buffer containing appropriate dose of test compound. It was followed by incubation for 15 minutes at room temperature (~22°C).

3. Wash: Residual Rb⁺ and compound were removed by four successive washes with 200 µl of SPA-Wash Buffer.

4. Cell Lysis: Intracellular samples were obtained by whole cell lysis with the application of 200 µl Lysis Solution.

5. Analysis: The level of Rb⁺ in the intracellular samples was measured by Aurora Biomed’s ICR8000™ using flame atomic absorption spectroscopy.

Results

1. Ready to use cells: The Cor.At® cardiomyocytes were observed to be ready to use cells for the assay after 3 to 4 days of maintenance.

2. Expression of Na⁺, K⁺ ATPase isoform: The Rb⁺ uptake profile observed in the assay indicated expression of this isoform in these cardiomyocytes (Figure 1) showing maximum uptake of Rb⁺ in about 80 minutes, with an exponential uptake seen from 0 to 50 minutes. Thus these cells can be employed for screening compounds in assays for 15 minutes to meet the demands of a HTS assay.
3. **Potency of standard blocker of Na\(^+\), K\(^+\) ATPase:** A complete block of Na\(^+\), K\(^+\) ATPase isoform was observed with 100 µM of digitoxin. The digitoxin was determined to have an IC\(_{50}\) value of 6.82 µM (Figure 2). The potency of digitoxin also displays a similar relation between mouse and Chinese hamster Na\(^+\), K\(^+\) ATPase isoform\(^5\).

**Figure 2.** Potency of Cor.At\(^\circledR\) cardiomyocyte Na\(^+\), K\(^+\) ATPase isoform displaying IC\(_{50}\) value for digitoxin.

**References**


**Conclusion**

The data suggest that the Cor.At\(^\circledR\) cardiomyocytes can be used as a predictive model for the identification of modulators of Na\(^+\), K\(^+\) ATPase in its original cardiac environment when using Rb Uptake Assay and ICR8000™.