Cell4Pharma Vesicular Transport Assay

Brief general protocol

Background

With the Cell4Pharma Vesicular Transport Assay substrate determination, inhibition studies and several kinetic studies can be performed. Transport can be measured radioactive, fluorescent or analytical (LCMS).

Necessary equipment:

- Plate Shaker
- Water bath
- MultiscreenHTS-Vacuum Manifold filtration device (Merck Millipore)
- Multichannel pipets (advised)
- Scintillation counter, fluorescent detector or LC-MS/MS

Necessary disposables:

- V-shape 96-wells incubation plates (651101, Greiner-bio-one) (make hole in corner to remove air and get better water contact)
- Filter plates
  - o PVDF (MSHVN4B50, Merck Millipore)
  - o Glass Fiber (MSFBN6B50, Merck Millipore)

Radioactive:

- Punching tips (MADP19650, Merck Millipore)
- Counting vials
- Topcount adapter, **for 96-wells counting only** (MSTPCWH50, Merck Millipore)

Fluorescent:

- Black flat bottom 96-wells plate (655096, Greiner-bio-one)

Analytical:

- Flat bottom 96-wells plate (655101, Greiner-bio-one)

Chemicals:

- Scintillation fluid (6013199, Perkin Elmer)

Buffers/Solutions:

- Cell4Pharma Stop/Wash buffer (2x)
- Cell4Pharma assay buffer (2x): 500 mM Sucrose, 20 mM Tris-HEPES, pH 7.4
- $MgCl_2$, 500 mM
- AMP, 100 mM pH 7.4
- ATP, 100 mM pH 7.4

Assay design advice:

- Performing incubations in triplicate
- For every test condition, dedicate three wells for AMP-containing buffer (background signal) and three wells for ATP-containing buffer. The difference between AMP- and ATP-containing conditions represents ATP-dependent uptake.

Info@Cell4Pharma.com
Kloosterstraat 9, 5349 AB, Oss Pivot Park, The Netherlands
www.cell4pharma.com

Protocol:

1. Prepare substrate mixture according to the following scheme:

Table 1: Substrate mixture (for multiple samples prepare a master mix)

Mixture for each well:		
Cell4Pharma assay buffer 2x	12.5	µl
Cell4Pharma MgCl2 (500 mM)	0.6	µl
Substrate	x *	µl
Inhibitor	x *	µl
Cell4Pharma AMP or ATP (100 mM)	1.2	µl
MilliQ	x *	µl
	Total 25	µl

Maximum final DMSO concentration 1 %

2. Place the v-shaped 96-wells plate on ice.
3. Thaw the vesicles at 37°C for 1 minute and place on ice.
4. Pipet 5 µl vesicles at the bottom of the well.
5. Add 25 µl/well ice cold substrate mixture prepared according to table 1 (mix on shaker for 10 sec).
6. Incubate the plate in a 37°C water bath for x minutes *
7. Dilute the original Cell4Pharma Stop/Wash buffer 2X first and stop the reaction by transferring the plate on ice and immediately add 150 µl ice-cold diluted Cell4Pharma Stop/Wash buffer.
8. Prewet the 96-wells filter plate # with 200 µl Cell4Pharma Stop/Wash buffer and apply vacuum to remove buffer just before transferring the samples.
9. Transfer the samples to the filter plate and wash twice with 200 µl Cell4Pharma Stop/Wash buffer by applying vacuum. Be sure the filters are dry after the second wash step.
10. The following step is dependent on the method of sample analysis:

a. Radioactivity counting in vails: remove plastic bottom underneath the filter plate and transfer filters using punching tips and add scintillation liquid
b. 96-wells radioactivity counting: carefully remove plastic bottom underneath the filter plate, place a topcount adapter and add scintillation liquid
c. Analytical measurements: place a flat-bottom 96-wells plate in the vacuum device, extract the samples with solvent # by applying vacuum.
d. Fluorescent measurements: place a black flat-bottom 96-wells plate in the vacuum device, extract the samples with solvent # by applying vacuum.

* Transporter dependent.
# Substrate dependent.

More detailed protocols including transporter-specific specifications are available.

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