Vesicles

Vesicular transport assay

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Vesicular transport assay

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).

Equiment:

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

- SV-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 measurement:

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

Fluorescent measurement:

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

Analytical measurement:

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

Buffers/Solutions:

- Scintillation fluid (6013199, Perkin Elmer)
- Cell4Pharma Stop/Wash buffer (2x)
- Cell4Pharma assay buffer (2x): 500 mM Sucrose, 20 mM Tris-HEPES, pH 7.4
- MgCl2, 500 mM
- AMP, 100 mM pH 7.4
- ATP, 100 mM pH 7.4

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Assay suggestions:

- 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

Preparation:

- Prepare substrate mixture according to the following scheme per well:

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 concentration of DMSO 1%

Procedure:

- Place the v-shaped 96-wells plate on ice.
- Thaw the vesicles at 37°C for 1 minute and place on ice.
- Pipet 5 µl vesicles at the bottom of the well.
- Add 25 µl/well ice cold substrate mixture prepared earlier (mix on shaker for 10 sec).
- Incubate the plate in a 37°C water bath for x minutes (transporter dependent)
- 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
- Prewet the 96-wells filter plate # with 200 µl Cell4Pharma Stop/Wash buffer and apply vacuum to remove buffer just before transferring the samples.
- 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.
- **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 (substrate dependant) by applying vacuum.
- **d.** Fluorescent measurements: place a black flat-bottom 96-wells plate in the vacuum device, extract the samples with solvent (substrate dependant) by applying vacuum.