Compound treatment in ioOligodendrocyte-like cells

ioCells™

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2.0

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1. Introduction

1.1. Introduction to the protocol

This protocol, developed and optimised at bit.bio, is a recommendation for screening small molecules using ioOligodendrocyte-like cells (cat no io1028) in a 96-well plate format.

ioOligodendrocyte-like cells provide a robust and consistent model for investigating compounds that modulate oligodendrocyte maturation and could affect myelination, making them ideal for drug discovery workflows targeting neurodegenerative and demyelinating diseases, such as multiple sclerosis. The assay is designed for image-based readouts to quantify markers of maturation, like myelin basic protein (MBP), and to assess cell proliferation. The streamlined workflow allows treatment to begin on day 1 post-thaw, with final analysis on day 8.

To demonstrate this compound treatment protocol, this guide uses TASIN-1 as a positive control. TASIN-1, a small molecule inhibitor of cholesterol biosynthesis, is a known pro-myelinating agent [1] and is therefore expected to increase the expression of MBP in ioOligodendrocyte-like cells in a dose-dependent manner.

The recommended treatment schedule begins on day 1 post-thaw with a full media change containing the compound at the final concentration. To maintain consistent exposure, the compound is replenished every two days via a half-media change, again using the final concentration.

Prior to testing novel molecules, we strongly recommend performing the assay with TASIN-1 to ensure familiarity with this protocol. We will anticipate that optimisation of experimental conditions (e.g., vehicle control, concentration range) may be required for your specific compounds of interest. Throughout this protocol, in addition to the developed protocol, we provide some guidelines and recommendations for optimisation steps.

1.2. Applicable ioCells Products

- ioOligodendrocyte-like cells (io1028S)

1.3. Recommendations for experimental plan

For support regarding cell culture of ioOligodendrocyte-like cells, please refer to the user manual. If you need assistance, visit www.bit.bio/support-hub.

- Before starting, read the complete ioOligodendrocyte-like cells user manual to prepare the necessary reagents and understand the complete cell culturing process.
- If testing compounds for the first time, consider testing a broad range of concentrations.
- bit.bio recommends starting the treatment on day 1 post-thaw. This longer period of compound treatment is favourable to maximise the compounds' effects at day 8, followed by readout of choice.
- When starting treatment on day 1, compounds should be introduced with a full media change at 1X concentration. After

initiating the treatment, compounds are replenished every second day with a half-media change using 1X concentration.

- Alternatively, compound treatment can also be started on day 3 or day 5 post-thawing. For those shorter treatment times, we recommend adding compounds at 2X concentration with a half-media change.
- Include a vehicle only control in your experiment, this refers to the solvent in which the test compounds are dissolved in.
- ioOligodendrocyte-like cells tolerate DMSO (as a vehicle) concentration of up to 0.5%. It is strongly recommended that the same vehicle concentration is used across all conditions.
- This protocol has been optimised and carried out in 96-well plates, testing proliferation and maturation effects of the compound Tasin-1.
- To mitigate plate effects caused by evaporation, fill the outermost wells of the 96-well plates with sterile PBS, rather than experimental testing conditions.

The following protocol recommends general guidelines. We encourage users to optimise the critical steps according to their experimental conditions.

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2. Materials and Equipment

2.1. Reagents and equipment for cell culture

Refer to ioOligodendrocyte-like cells User Manual for reagents and equipment required for plate coating, cell thawing, seeding and culture.

2.2. Application-specific reagents and equipment

2.2.1. Equipment

Material	Supplier	Cat no
µ-Plate 96 Well Black	IBIDI	89626-90
2,0 ml PlateOne® 96-Deepwell-Plate	Starlab	S1896-2110
Sterile V-bottom 96-well plate	Greiner	651261
Fluorescence Microscope	-	-

Table 1
Application-specific equipment.

2.2.2. Reagents

Material	Supplier	Cat no
DMSO	Sigma-Aldrich	D2650
Tasin-1	MedChemExp	HY-116572A

Table 2
Application-specific reagents.

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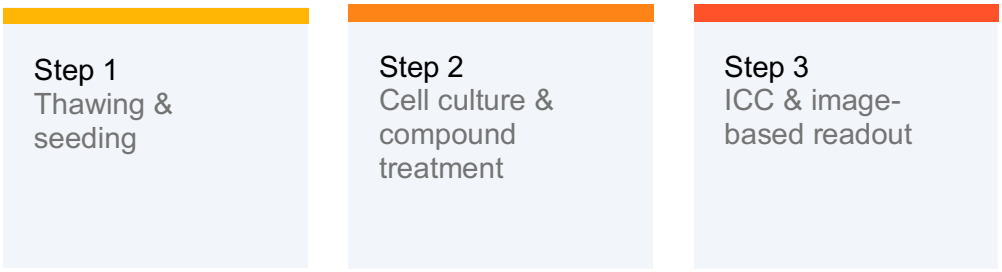
3. Protocol

This protocol has 3 steps:

Step 1: Thawing and seeding cells (as per user manual)
Step 2: Cell culture and compound treatment
Step 3: Immunocytochemistry (ICC) & image-based readout (as per ICC protocol)



3.1. ioOligodendrocyte-like cells thawing & seeding

Thaw and seed cells according to the protocol described in the ioOligodendrocyte-like cells user manual.

To mitigate plate effects through evaporation, fill the outermost wells of the 96-well tissue culture plate with PBS.

3.2. Compound treatment (starting at day 1, day 3 or day 5 post-thaw)

Before starting:

- Compound treatment of the ioOligodendrocyte-like cells is carried out alongside the regular media changes as described in the user manual (NPI-0013 UM V-02).
- Prepare comp:OM2 medium (as per the user manual) and warm it up to 37°C.
- Compound treatment can be started on day 1, day 3 or day 5 post thawing.
  - If starting treatment on day 1, small molecules are first introduced with a full media change at 1X concentration (see section 3.2.3.
  - If starting day 3 or day 5, small molecules are first introduced with a half media change at 2X concentration to achieve a final working concentration of 1X. (see section 3.2.4).
- Compounds are replenished every second day (48 hours) with a half-media change at 1X concentration.
- Depending on the testing compound concentration and final DMSO concentration, a two-step dilution of the compounds is recommended to ensure the final DMSO concentration is the same in all conditions.

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- bit.bio recommends one predilution in DMSO and one predilution in comp:OM2 media before preparing the final media at the final concentration required (refer to figure 1 for a visual schematic).

If possible, randomize conditions and replicates across the 96-well plate to mitigate plate location effects in the readouts.

Evaluated compound	Tasin-1
Compound stock concentration	10 mM
Vehicle	DMSO
Vehicle concentration	0.5%
Compound concentrations to be tested	500 nM, 250 nM, 125 nM, 62.5 nM, 31.25 nM, 15.63 nM
Plate format	96 well plate
Volume per well	200 µl
Replicates per condition	5
Treatment starting point	Day 1
Treatment ending point	Day 8

Table 3
Assay conditions and assumptions used throughout this protocol.

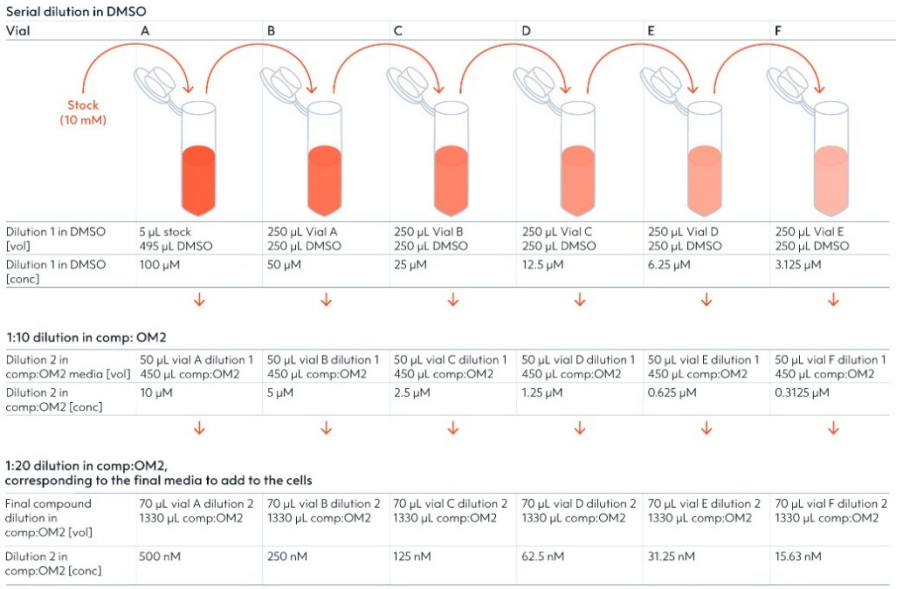

Figure 1
Overview of the dilution steps required to start a dose-response treatment of Tasin-1 on day 1. A serial dilution of compounds in DMSO is diluted in comp:OM2, before being diluted to the final working concentration in comp:OM2 to be added to the cells. Detailed instructions are provided in the following sections.

When preparing stock dilutions, we recommend preparing extra volume to account for standard pipetting losses. The volumes described in the tables below already account for this extra volume.

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3.2.1. Preparing dilution 1 in vehicle control

- Thaw the compound stock to room temperature.
- Prepare dilution 1 in DMSO (vehicle control) for each treatment condition (see table 4) including DMSO.
- Ensure the dilutions in DMSO are properly mixed before moving on to prepare dilution 2.

Always include a vehicle only control in your experiment, this refers to the solvent in which the test compounds are dissolved in. On this protocol, the vehicle control is DMSO.

Vial	Volume of compound (µL)	Volume of DMSO (µL)	Final concentration (µM)
Stock	-	-	10000
A	5 of stock	495	100
B	250 of A	250	50
C	250 of B	250	25
D	250 of C	250	12.5
E	250 of D	250	6.25
F	250 of E	250	3.125

Table 4
Preparation of compound dilution 1 in vehicle control.

3.2.2. Preparing dilution 2 in cell culture medium

- Dilute dilution 1 in fresh, pre-warmed comp:OM2 medium as per Table 6 to prepare dilution 2. The complete formulation of comp:OM2 medium is described in the user manual of ioOligodendrocyte-like cells.
- Include a dilution of DMSO in fresh, pre-warmed comp:OM2 to be used as vehicle control.
- Ensure the dilutions in cell culture media are properly mixed before moving on to prepare the final dilution.

Vial	Dilution 1 (µL)	Volume of comp:OM2 medium (µL)	Final concentration (µM)
A	50	450	10
B	50	450	5
C	50	450	2.5
D	50	450	1.25
E	50	450	0.625
F	50	450	0.3125

Table 5
Preparation of compound dilution 2 in cell culture media (comp:OM2 media).

- If starting the compound treatment on day 1, continue with section 3.2.3.

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- If starting the compound treatment on day 3 or day 5 continue with section 3.2.4.

3.2.3. For treatment from day 1:

Preparing final dilution to 1X concentration in comp:OM2 media

- To obtain the final treatment concentration (1X), further dilute dilution 2 in fresh, pre-warmed comp:OM2 media (refer to table 6).
- Exchange 100% of the cell culture media (200 µL per well) with the compound dilution at 1X concentration.
- Use an automated multichannel pipette at the slowest speed to mitigate mechanical stress on the cell layer.

Vial	Dilution 2 (µL)	Volume of comp:OM2 medium (µL)	Final concentration (nM)
A	70	1330	500
B	70	1330	250
C	70	1330	125
D	70	1330	62.5
E	70	1330	31.25
F	70	1330	15.63

Table 6
Preparation of final compound dilution to 1X concentration to start treatment on day 1, volumes account for 5 replicate wells (already including extra volume).

3.2.4. For treatment starting at day 3 or day 5:

Preparing final dilution to 2X concentration in comp:OM2 media

- To obtain the final treatment concentration (2X), further dilute dilution 2 in fresh, pre-warmed comp:OM2 media (refer to table 7).
- Exchange 50% of the cell culture media (100 µL per well) with the compound dilution in comp:OM2 at 2X concentration.
- Use an automated multichannel pipette at the slowest speed to mitigate mechanical stress on the cell layer.

Vial	Dilution 2 (µL)	Volume of comp:OM2 medium (µL)	Final concentration (nM)
A	70	630	1000
B	70	630	500
C	70	630	250
D	70	630	125
E	70	630	62.5
F	70	630	31.25

Table 7
Preparation of final compound dilution to 2X concentration to start treatment on day 3 or 5, volumes account for 5 replicate wells (already including extra volume).

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3.2.5. Replenishment of compounds throughout media changes

- Every 48 hours, aligning with the media changing schedule of the user manual, repeat sections 3.2.1 and 3.2.2 before preparing a 1X compound dilution for a half media-change (see table 8).
- Exchange 50% of the cell culture media (100 µL) with the compound dilution in comp:OM2 at 1X concentration.
- Use an automated multichannel pipette at slowest speed to mitigate mechanical stress on the cell layer.

Vial	Dilution 2 (µL)	Volume of comp:OM2 medium (µL)	Final concentration (nM)
A	35	665	500
B	35	665	250
C	35	665	125
D	35	665	62.5
E	35	665	31.25
F	35	665	15.63

Table 8
Preparation of final compound dilution to 1X concentration to replenish compound throughout cell culture length, volumes account for 5 replicate wells (already including extra volume).

3.3. Immunocytochemistry and readout

- Perform readout of choice at day 8 post-thaw.
  - If performing ICC readout, we recommend fixing and staining the cells according to bit.bio's recommended ICC protocol for ioOligodendrocyte-like cells.

4. Reference Data

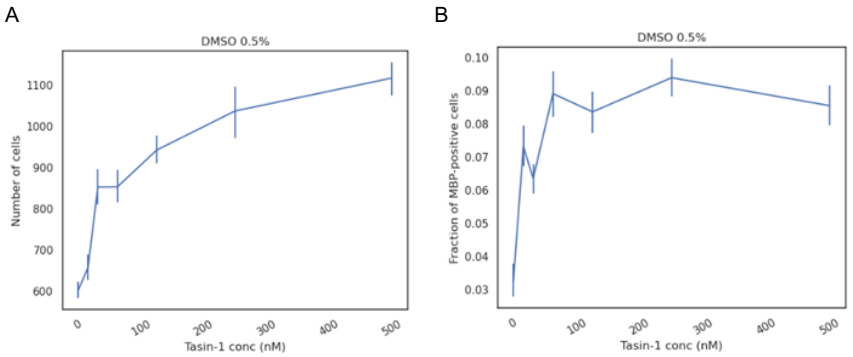

Figure 2
Quantification of the dose-dependent response of ioOligodendrocyte-like cells to Tasin-1, measured at day 8 post-revival. The treatment with Tasin-1 results in an increased number of cells in culture and moreover, an increase in the fraction of MBP-positive cells indicating enhanced cell maturation. **(A)** The graph shows the total number of cells, determined by DAPI counterstain. **(B)** The graph shows the fraction of cells positive for the mature myelin marker, Myelin Basic Protein (MBP). n= 5 replicates.

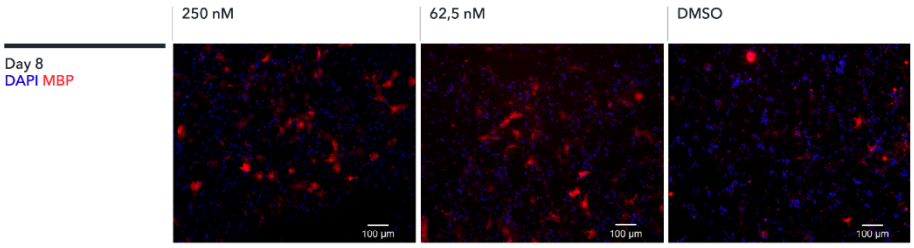

Figure 3
Representative immunofluorescent images of ioOligodendrocyte-like cells at day 8 post-revival, treated with 250 and 62,5 nM Tasin-1 and vehicle control (0.5% DMSO. Cells were stained for MBP (red), and the DAPI counterstain (blue). The expression of MBP increases with Tasin-1 treatment, confirming a dose-dependent enhancement of MBP expression.

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5. Troubleshooting

Issue	Possible reason	Possible solution
High variability in replicates of wells at the edge of the plate	Increased media evaporation in wells located at the edges of the plate	Leave 1-2 rows and columns at the plate edges filled with sterile PBS
Compound precipitation in cell culture media (visible turbidity or micro-precipitates)	The solubility of the compound in comp:OM2 media has been exceeded.	Test lower compound concentrations or consider increasing the DMSO concentration in media
Uneven cell distribution after seeding	Not complete cell distribution at the time of seeding	Gently cross-shake the plate once on the incubator shelf (back and forth, side to side, 2-3 times)

6. Related protocols and resources

- User manual of ioOligodendrocyte-like cells
- ICC of ioOligodendrocyte-like cells

7. References

Hubler Z, et al (2008). Accumulation of 8,9-unsaturated sterols drives oligodendrocyte formation and remyelination. Nature. doi: 10.1038/s41586-018-0360-3. PMID: 30046109. PMCID: PMC6423962

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