Astrocyte Reprogramming Reagent Kit (DFICBY)

Item No. 502887

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GENERAL INFORMATION

Materials Supplied

Item No.	Item Name	Quantity/Size	Storage
14408	Bucladesine (sodium salt)	1 vial/50 mg	-20°C
11018	Forskolin	1 vial/5 mg	-20°C
16165	ISX-9	1 vial/10 mg	-20°C
13122	CHIR99021	1 vial/10 mg	-20°C
11181	I-BET151	1 vial/1 mg	-20°C
10005583	Y-27632 (hydrochloride)	1 vial/5 mg	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Cell culture-grade DMSO

INTRODUCTION

Description

The components of this kit can be mixed to produce the small molecule cocktail DFICBY. When mixed with appropriate cell culture media, DFICBY promotes reprogramming of astrocytes into neuronal cells with high conversion efficiency.[1] Forskolin is a cyclic AMP (cAMP) agonist, bucladesine and ISX-9 are promoters of neural stem cell differentiation, CHIR99021 is an inhibitor of glycogen synthase kinase 3 (GSK-3) that regulates Wnt/β-catenin signaling, I-BET151 is a bromodomain inhibitor that disrupts fibroblast-specific programming, and Y-27632 is an inhibitor of Rho-associated kinase (ROCK) that increases reprogramming efficiency.[1-2] This optimized small molecule cocktail demonstrated improved performance over the previously developed FICBY cocktail for astrocyte reprogramming.[2] DFICBY disrupts fibroblast-specific programming, activates endogenous expression of neuronal-specific genes, and converts astrocytes to functional neuronal cells.

Each component in this kit is provided separately, and instructions are included to reconstitute these reagents for direct dilution into cell culture media. The kit is intended to supplement up to 1 L of culture media when following the supplied instructions. Some reagents may be provided in excess and may be properly disposed of after cell media preparation. Prior to use, reagents must be stored as indicated at -20°C.

PREPARATION

Reagent Preparation

Preparation of Individual Stock Solutions

1. Prepare a 1,000 mM (10,000X) stock solution of bucladesine by adding 102 µl of sterile DMSO to the vial and vortexing until fully dissolved. The bucladesine solution may be diluted in cell culture media to a final concentration of 100 µM.
2. Prepare a 100 mM (10,000X) stock solution of forskolin as by adding 122 µl of sterile DMSO to the vial and vortexing until fully dissolved. This forskolin solution may be diluted in cell culture media to a final concentration of 10 µM.
3. Prepare a 400 mM (10,000X) stock solution of ISX-9 by adding 107 µl of sterile DMSO to the vial and vortexing until fully dissolved. This ISX-9 solution may be diluted in cell culture media to a final concentration of 40 µM.
4. Prepare a 200 mM (10,000X) stock solution of CHIR99021 by adding 108 µl of sterile DMSO to the vial and vortexing until fully dissolved. This CHIR99021 solution may be diluted in cell culture media to a final concentration of 20 µM.
5. Prepare a 20 mM (10,000X) stock solution of I-BET151 by adding 120 µl of sterile DMSO to the vial and vortexing until fully dissolved. This I-BET151 solution may be diluted in cell culture media to a final concentration of 2 µM.
6. Prepare a 100 mM (10,000X) stock solution of Y-27632 by adding 156 µl of sterile DMSO to the vial and vortexing until fully dissolved. This Y-27632 solution may be diluted in cell culture media to a final concentration of 10 µM.
7. Upon addition of all reagents, sterile filtering the final media formulation is recommended prior to use.

NOTE: Concentrated stock solutions may be stored at -20°C for up to one month, avoiding multiple freeze-thaw cycles. Sterile filtration may be required upon dilution in media.

RESOURCES

References

1. Ma, Y., Xie, H., Du, X., *et al. In vivo* chemical reprogramming of astrocytes into neurons. *Cell Discov.* **7**, 13 (2021).
2. Li, X., Zuo, X., Jing, J., *et al.* Small-molecule-driven direct reprogramming of mouse fibroblasts into functional neurons. *Cell Stem Cell* **17**, 195-203 (2015).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.