Primary Human Hepatocytes Maintenance Kit (5C)

Introduction

The liver is a very important organ in the human body, which plays the functions of metabolism, detoxification, immunity and so on. Hepatocytes make up about 80-90% of the liver and are the main bearers of liver function. The isolation and culture of primary hepatocytes is one of the most important aspects of mimicking liver diseases and understanding their molecular mechanisms. However, freshly isolated hepatocytes can only be maintained in vitro for about 10-14 days, during which the function of hepatocytes will be continuously lost, and then they will become fibrous mesenchymal cells and eventually apoptosis. This is inconvenient mimicking liver disease.

In 2019, Hongkui Deng's group published a paper "Long-term functional maintenance of primary human hepatocytes in vitro" in the *Science*, in which they found five compounds that can effectively prolong the time of in vitro culture of primary hepatocytes, and named the combination of these five compounds 5C. These five compounds are Forskolin, SB431542, DAPT, IWP-2 and LDN193189, and their specific information is shown in Table 1, which mainly maintain hepatocytes by affecting related signaling pathways such as differentiation and proliferation. They found that the addition of these 5 compounds to the primary hepatocyte culture process could extend the culture time to 4 weeks and effectively maintain the shape of hepatocytes and the stable expression of related genes.

Primary Human Hepatocytes Maintenance Kit (5C) provides ready-to-use 5C combination reagents, all of which are supplied as stock solutions dissolved in high-purity DMSO. According to the recommended concentration in this manual, this kit can prepare 1 L of culture medium.

Compounds	CAS Number	MW	Target
Forskolin	66575-29-9	410.50	cAMP activator
SB431542	301836-41-9	384.39	TGF-β/Smad inhibitor
DAPT	208255-80-5	432.46	Notch inhibitor
IWP-2	686770-61-6	466.60	Wnt inhibitor
LDN193189	1062368-24-4	406.48	BMP inhibitor

Table 1: Basic information on each compound in the 5C combination

Components and Storage

Components	K2713-1 Package
Forskolin (20 mM)	1 mL
SB431542 (10 mM)	1 mL
DAPT (10 mM)	100 μL
IWP-2 (5 mM)	100 μL
LDN193189 (1 mM)	100 μL
Store the kit at -20°C, stable for at least 1 year.	

Protocol

1. Warm the reagent to room temperature, then centrifuge at 2000-10000 rpm for seconds to collect all the liquid to the bottom of the tube before opening the lid and using it.
2. Refer to the following conditions or other references to dilute the reagents in the medium for culturing primary hepatocytes, or explore the optimal working concentration by yourself.

Reagents	Dilution Ratio	Working concentrate
Forskolin (20 mM)	1000	20 μM
SB431542 (10 mM)	1000	10 μM
DAPT (10 mM)	10000	1 μM
IWP-2 (5 mM)	10000	0.5 μM
LDN193189 (1 mM)	10000	0.1 μM

Note

1. All reagents in this kit are supplied as stock solutions dissolved in high-purity DMSO.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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