SILAC-RPMI (3plex) Kit

Catalog Number: SM202109

(Version 1.0)

Description

SILAC (*Stable Isotope Labeling with Amino acids in Cell culture*) is a powerful quantitative proteomic method to identify and quantify the relative changes of protein abundance in complex protein samples. This approach utilizes the *in vivo* metabolic incorporation of "heavy" ^{13}C- or/and ^{15}N-labeled amino acids (Lysine, Arginine, etc.) into proteins during cell culture, introducing the specific "MS tag" on proteins in comparison with the unlabeled counterpart, which enables the mass spectrometry (MS) to comprehensively identify, characterize, and quantify the proteins.

Contents

Product	Cat. No.	Contents	Storage
SILAC-RPMI (3plex) Kit	SM202109	1×500ML (K0R0)	4°C
		1×500ML (K4R6)	4°C
		1×500ML (K8R10)	4°C
		3×50ML D-FBS (DF-50)	-20°C

SILAC-RPMI (3plex) Kit is designed to compare **three** groups of samples using SILAC approach. K and R amino acids added into each medium are listed as below:

K0R0	K4R6	K8R10
Lysine (K0)	(4,4,5,5-D4)-Lysine (K4)	$^{13}C_6$, $^{15}N_2$-Lysine (K8)
Arginine (R0)	$^{13}C_6$-Arginine (R6)	$^{13}C_6$, $^{15}N_4$-Arginine (R10)

For experimental design, culture of group 1 in **K0R0** medium (recommend as control group, Light), group 2 in **K4R6** (Medium), and group 3 in **K8R10** (Heavy), respectively. In downstream MS analysis, three MS1 pairs will be observed in K- (0, 4, 8 Dalton) or R (0, 6, 10 Dalton)-containing peptides, enabling the relative quantification of protein expression level or enriched level (interaction profiling) among three groups.

Usage & Application

1. Before use, add 10% (v/v) of D-FBS (DF-50) into **K0R0**, **K4R6**, and **K8R10**

media (complete media) respectively.

2. Use **K4R6 and K8R10** complete media to culture cells over six passages, extract proteins, and check the SILAC labeling efficiency by mass spectrometry, respectively.
3. Cryopreservation of **K4R6 and K8R10**-labeled cells with the standard protocol for long-term use.
4. For primary cells and cell lines sensitive to dialyzed FBS, SILAC method is not recommended.

- **Troubleshooting**

Problem	Cause	Solution
1. Cells are not viable or grow poorly. 2. Cell morphological changes.	Probably due to dialyzed FBS. Dialyzed FBS lacks some small molecules important for cell growth.	1. Change cell lines insensitive to dialyzed FBS. 2. Increase the dialyzed FBS up to 15-20%. 3. Try and test the dialyzed FBS from other vendors.