SILAC-RPMI (3plex) Kit

Catalog Number: SM202109

(Version 1.5)

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 by MS (mass spectrometry) [1]. This approach utilizes the *in-cell* 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.	Content	Item code	Size	Quantity	Storage
SILAC-RPMI (3plex) Kit	SM202109	K0R0	SCR01	500mL	1	4°C
		K4R6	SR04	500mL	1	4°C
		K8R10	SR03	500mL	1	4°C
		D-FBS	DF-50	50mL	3	-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 **K0R0**, **K4R6**, and **K8R10** complete media to culture cells over four to six passages in parallel.
3. Cryopreservation of **K0R0-** , **K4R6-**, and **K8R10**-labeled cells using the corresponding SILAC-complete media with the standard protocol for long-term use.
4. Use ***Co-IP/Pull-down In-solution Trypsin Digestion (ISD) Kit*** (Imultiomics, #MG04) to prepare digested peptides for MS check of SILAC labeling efficiency.
5. For primary cells and cell lines sensitive to dialyzed FBS, SILAC method is not recommended.

Troubleshooting

Problem	Cause	Solution
1. Cells grew poorly or changed in morphology.	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.
2. Incomplete SILAC labeling	1. Labeling passages were insufficient. 2. Cells were contaminated during the labeling process. 3. In rare occasion, Arginine was converted to Proline.	1. Increase labeling passages. 2. Change cell lines.

References

1. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M: **Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics**. *Mol Cell Proteomics* 2002, **1**(5):376-386.