SILAC-RPMI (K8R10) Kit

Catalog Number: SM202108

(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

Name	Cat. No.	Content	Item code	Size	Quantity	Storage
SILAC-RPMI (K8R10) Kit	SM202108	K0R0	SCR01	500mL	1	4°C
		K8R10	SR03	500mL	1	4°C
		D-FBS	DF-50	50mL	2	-20°C

SILAC-RPMI (K8R10) Kit is designed to compare two groups of samples using SILAC approach. The formulation of **K8R10** is identical to that of **K0R0** except that **$^{13}C_6$, $^{15}N_2$-Lysine (K8)** and **$^{13}C_6$, $^{15}N_4$-Arginine (R10)** replace the **Lysine (K0)** and **Arginine (R0)**. Because of their identity, **K0R0** is the ideal control medium for **K8R10**.

When two groups of cells were cultured in parallel, the additional **8** or **10 Dalton** of "MS tag" would be introduced to the Lysine and Arginine residues respectively on the proteins of cell cultured in **K8R10** medium compared to that of maintained in **K0R0** medium, and the "MS tag" is the key for protein relative quantitation by downstream mass spectrometry analysis.

Usage & Application

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

2. Use **K0R0** and **K8R10** complete media to culture cells over four to six passages in parallel.
3. Cryopreservation of **K0R0-** 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 K0R0- and K8R10-labeled 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.