

SILAC

B060

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Taken from: Lößner et al.; “Quantitative Proteomics Identify Novel miR-155 Target Proteins”; PLOS One; 2011

For SILAC analysis, SILAC D-MEM media supplemented with 10% dialyzed FBS and either 100 mg/L $^{12}\text{C}_6$ -L-arginine and $^{12}\text{C}_6$ -L-lysine or $^{13}\text{C}_6$ -L-arginine and $^{13}\text{C}_6$ -L-lysine (Invitrogen Corporation, Carlsbad, USA) as well as 200 mg/L $^{12}\text{C}_5$ -L-proline (Promega Corporation, Madison, USA) were used. HEK293T cells were serially passaged (2×10^6 cells/10 cm dish) and grown for five doublings to ensure full incorporation of labelled amino acids. Subsequently, the cells were transfected with either 2 μg of the pCMX-miR-155 construct or the empty plasmid. The cells were harvested 48 h after transfection and counted using a cell counter (Vi-CELL XR; Beckman Coulter, Fullerton, USA). Aliquots of cells were mixed in a one to one ratio, washed two times with ice-cold phosphate-buffered saline (PBS), shock-frozen in liquid nitrogen for storage at -80°C . A technical and biological replicate of the same experiment was done with reverse labelled samples