Silac media and labelling protocol

AP + MQ 19.10.2016

Medium preparation

Reagents

Product	***Comment***	***mw***	***supplier***	***Product number***	***Provided by PAF to customers***
Special DMEM or RPMI for SILAC W/O Arg, Lys	To be ordered by customer	-	Thermofisher / Pierce	On web site, search for "silac kits & reagents" or see below article number.)	N
Dialysed FBS	PAF filters it at reception	-	Gibco # (Sigma)	26400-036	Y
Penicilin/Streptomycine 10000 IU/ml			Amimed	# 5-51F00-H	N
L-Glutamine 100X (Gibco #25030).	Mostly present in medium but some people prefer to add some extra	-			N
L-Lysine:2HCl	Light	219.11	Sigma	L5751	Y
L- Lysine:2HCl (**K+6**) (U-13C6 99%)	Heavy	225.07	CIL	CLM-2247-...	Y
L- Lysine:2HCl (**K+8**) (U-13C6 99%,15N2 99%)	Heavy	227.05	CIL	CNLM-291-...	Y
L-Arginine:HCl	Light	210.66	Sigma	A6969	Y
L-Arginine:HCl (**R+10**) (U-13C6 99%; U-15N4 99%)	Heavy	220.59	CIL	CNLM-539-...	Y
L-Proline	Light ; added in excess to suppress Arg→Pro conversion in some cell lines		Sigma	P5607-25G	Y

Procedure:

- Prepare stock solution for each AA in PBS1X, keep non sterile aliquots at -80C (can be thawed a few times)
- Dialysed FBS: in order to remove small debris, filtrate 1st thought 150mm folded filter, then 45um Filtropur *(Sarstedt #83.1826)* make 5, 10 and 25 ml aliquots and keep non sterile aliquots at -20C

For 100ml DMEM for SILAC final volume *(SILAC DMEM #89985 Thermo Scientific)*

ADD TO 89 ml of special medium:

	STOCK mg/ml	FINAL CONC. mg/L	LIGHT volume nedded		HEAVY volume nedded	
Lysine 0 or K0	50	150	**300**	ul	--	--
Lysine +6 or K+6	150	150	--	--	**100.0**	ul
Arginine 0 or R0	50	50	**100**	ul	--	--
Arginine +10 or R+10	50	50	--	--	**100**	ul
Proline 0 (excess)	50	200	**400**	ul	**400**	ul
Dialyzed FBS			**10**	ml	**10 ml**	ml
Pen / Strept			**0.5**	ml	**0.5**	ml

For 100 ml RPMI for SILAC final volume *(SILAC RPMI 1640 #89984 Thermo Scientific)*

ADD TO 88 ml of special medium:

	STOCK mg/ml	FINAL CONC. mg/L	LIGHT volume nedded		HEAVY volume nedded	
Lysine 0 or K0	50	100	**200**	ul	--	--
Lysine +6 or K+6	150	100	--	--	**66.7**	ul
Arginine 0 or R0	50	100	**200**	ul	--	--
Arginine +10 or R+10	50	100	--	--	**200**	ul
Proline 0 (excess)	50	180	**360**	ul	**360**	ul
Dyalized FBS			**10**	ml	**10**	ml
Pen / Strep			**0.5**	ml	**0.5**	ml

- Mix all non sterile AA + serum + Antibiotics needed and filter through a 22um Filtropur *(Sarstedt # 83.1826.001)* filter directly into the medium.
- In order to remove rests of mix from the filter, rinse it using 1 ml of medium

Cell culture

- Grow cells using light or heavy Special DMEM or RPMI cell culture medium
- To be 100% labeled cells must go through at least 5 or 6 cell cycle division
- During all cell culture procedure (trypsinization, freezing etc….) use only dialysed serum and light or heavy labeled DMEM or RPMI
- Keep only a small volume of culture at the beginning. This allows not to use too much medium and also one can dilute cells more in the first passages

To Harvest cells

- Important, cells must be in exponential phase to be harvested
- Harvest cells as usual
- Transfer cells in 15 ml tube
- Spin as usual
- Remove and discard cell culture medium
- Wash cells 2x with 10ml PBS 1x
- Resuspend cell in 1ml PBS 1x (3rd wash), transfer cells into a 1.5 ml Eppendorff tube
- Centrifuge 2 min 2'000 rpm in a microcentrifuge
- Remove completely PBS 1x

Cell lysis

Depending on the type of analysis that follows, lysis can be done with two methods. The choice of lysis has to be discussed in advance.

1. Lysis in FASP buffer

FASP Lysis buffer : 4% SDS, 0.1M DTT, 100mM Tris pH7.5

- Tap tube with the fingers to loosen up the cell pellet; this is important to expose cells evenly to the lysis buffer and avoid formation of an insoluble aggregate
- Resuspend quickly cells with a certain volume (see below) of lysis buffer (try to pipet up/down 1or 2x but will get very viscous)
- Heat 5min 95C
- Sonicate 3x 5 sec with a tip sonicator to shear DNA
- Centrifuge 10min 13'000rpm
- Transfer supernatant to new tubes

Volume of lysis buffer to use: we typically use 200-300ul buffer / 10e7 cells. If much smaller amounts of cells are used, proportionally larger buffer volumes can be used (i.e. for 10e6 cells use 25 -30 ul) to have final workable volumes.

Note: You can keep aliquots at-20C and defrost them a few times. Samples can be heated up to 95C to make sure there are not aggregates/precipitates

2. Lysis in Urea buffer

8M Urea lysis buffer : 8M Urea, 20 mM Hepes, pH 7.2, Protease inhibitors (Roche or others), Phosphatase inhibitors (optional (Roche or others))

- Tap tube with the fingers to loosen up the cell pellet; this is important to expose cells evenly to the lysis buffer and avoid formation of an insoluble aggregate
- Resuspend quickly cells with a certain volume (see below) of lysis buffer (try to pipet up/down 1or 2x but lysate will get very viscous)
- Sonicate 3x 5 sec with a tip sonicator (in case you need we have one in the Biochimie TP room)
- Centrifuge 10min 13'000rpm
- Transfer supernatant and freeze it at -20 or -80C
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!! **Never warm up protein extracts in 8M Urea** (not even for loading on SDS gels) as this results in protein carbamylation.

Volume of lysis buffer to use: we typically use 300ul / 10e7 cells. If much smaller amounts of cells are used, proportionally larger buffer volumes can be used (i.e. for 10e6 cells use 25 -30 ul) to have final workable volumes.

Note: minimize freeze/thaw cycles in this buffer as there is the danger of protein aggregation and precipitation