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 at reception and aliquots it	-	Gibco # SIGMA ??	26400-036	Y
Penicilin/Streptomycine 10000 IU/ml			Amimed	# 5-51F00-H	N
L-Glutamine 100X	Mostly present in medium but some people prefer to add some extra	-	Gibco	#25030	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 (*done by PAF already*)

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 needed		HEAVY volume needed	
Lysine 0 or K0	50	150	**300**	ul	--	--
Lysine +6 or +8 (K+6/ K+8)	150	150	--	--	**100.0**	ul
Arginine 0 or R0	50	50	**100**	ul	--	--
Arginine +6 or +10 (R+6/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 +8 (K+6/ K+8)	150	100	--	--	**66.7**	ul
Arginine 0 or R0	50	100	**200**	ul	--	--
Arginine +10 or R+10 (R+10)	50	100	--	--	**200**	ul
Proline 0 (excess)	50	180	**360**	ul	**360**	ul
Dialyzed FBS			**10**	ml	**10**	ml
Pen / Strept			**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 (and wether or not to lyse at all…) 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