

PROTOCOL: GCP Lysis Buffer and Additives Prep (LBAP)

Purpose

Prepare reagents that can be stored long-term for the Global Chromatin Profiling Assay

Materials

- HPLC-grade water {LBAP-M01}
- Dimethylsulfoxide (DMSO) {LBAP-M02}
- Sucrose {LBAP-M03} [Sigma, S9378]
- KCl {LBAP-M04} [Sigma, 60128]
- NaCl {LBAP-M05} [Fluka, 71738]
- Trizma-HCl {LBAP-M06} [Sigma, T5941]
- Trizma Base {LBAP-M07} [Sigma, T6066]
- MgCl₂ {LBAP-M08} [Sigma, M8266]
- CaCl₂ {LBAP-M09} [Sigma, 1016]
- Sodium Butyrate {LBAP-M10} [Sigma, B5887]
- AEBSF {LBAP-M11} [Calbiochem, 101500]
- Microcystin LR {LBAP-M12} [Calbiochem, 475815]
- NP40 {LBAP-M13} [USB Corporation, 19628]
- 250 mL Glass Bottles with Solids Screw Top {LBAP-M14}
- 15 mL Conical Vial {LBAP-M15}
- 500 mL glass graduated cylinder {LBAP-M16}
- Magnetic stir bar {LBAP-M17}
- 500 mL vacuum filtration unit with storage bottle {LBAP-M18} [VWR, 16211-064]

Assets

- Mass Balance {LBAP-A01}
- Bench Vortex {LBAP-A02}

Reagent Mixes

ID	Name	Step	Composition	Stock Volume	Use
LBAP-ADD0 1	1M KCl	HAE	74.55 mg/mL in {LBAP-M01}	200 mL	Additive to Nucleus Buffer
LBAP-ADD0 2	1M NaCl	HAE	58.44 mg/mL NaCl in {LBAP-M01}	200 mL	Additive to Nucleus Buffer
LBAP-ADD0 3	1M Tris pH 7.5	HAE	0.83 mol {LBAP-M06} and 0.17 mol {LBAP-M07} in {LBAP-M01}	250 mL	Additive to Nucleus Buffer
LBAP-ADD0 4	0.1M MgCl ₂	HAE	95.21 mg/mL {LBAP-M08} in {LBAP-M01}	100 mL	Additive to Nucleus Buffer

LBAP-ADD05	1M CaCl ₂	HAE	110.98 mg/mL {LBAP-M09} in {LBAP-M01}	10 mL	Additive to Nucleus Buffer
LBAP-ADD06	2.27M sodium butyrate	HAE	250 mg/mL in {LBAP-M01}	4 mL	Additive to Lysis Buffer
LBAP-ADD07	250 mM AEBSF	HAE	59.925 mg/mL in {LBAP-M01}	8.344 mL	Additive to Lysis Buffer
LBAP-ADD08	5 uM Microcystin LR	HAE	0.497 mg/mL in {LBAP-M02} (500 uM stock bottle)	1.005 mL	Additive to Lysis Buffer
LBAP-ADD09	10% NP40	HAE	10% {LBAP-M13} in {LBAP-M01}	200 mL	Additive to Lysis Buffer
LBAP-BUF01	Nucleus Buffer	HAE	250mM {LBAP-M03}, 60mM {LBAP-ADD01}, 15mM {LBAP-ADD02}, 15mM {LBAP-ADD03}, 5mM {LBAP-ADD04}, 1mM {LBAP-ADD05}	500 mL	Base to the Lysis Buffer

Preparation of Additives and Nucleus Buffer

LBAP-ADD01: 1M KCl

- 1) Weigh out at least 14.91 g KCl {LBAP-M04} to make at least 200 mL 1M KCl. Add reagent to 250 mL glass bottle {LBAP-M14}
- 2) Calculate HPLC-grade water to add {LBAP-M01} by dividing amount weighed by 74.55 mg
 - Amount Weighed: _____ mg
 - Divide by: 74.55 mg/mL
 - Volume {LBAP-M01} to add: _____ mL
- 3) Add calculated amount of {LBAP-M01} to bottle containing reagent. Vortex till reagent is dissolved

LBAP-ADD02: 1M NaCl

- 1) Weigh out at least 11.69 g NaCl {LBAP-M05} and pour into a pre-conditioned 250 mL glass bottle {LBAP-M14}.
- 2) Calculate HPLC-grade water {LBAP-M01} to add by dividing amount weighed by 58.44 mg
 - Amount weighed: _____ mg
 - Divide by: 58.44 mg/mL
 - Volume {LBAP-M01} to add: _____ mL
- 3) Add calculated amount of {LBAP-M01} to bottle containing reagent. Vortex till reagent is dissolved

LBAP-ADD03: 1M Tris pH 7.5

- 1) Add 32.7g Trizma-HCl and 5.15g Trizma-base to 250 mL glass bottle {LBAP-M14} pre-conditioned with {LBAP-M01}
- 2) Bring up to 250 mL with {LBAP-M01}
- 3) Adjust to pH 7.5 with 1M Trizma-HCl and 1M Trizma-base as needed

LBAP-ADD04: 0.1M MgCl₂

- 1) Weigh out at least 0.952 g MgCl_2 for 100 mL 0.1M MgCl_2 and pour into a pre-conditioned 250mL glass bottle {LBAP-M14}
- 2) Calculate HPLC-grade water {LBAP-M01} to add by dividing amount weighed by 9.521 mg
 - Amount weighed: _____ mg
 - Divide by: 9.521 mg/mL
 - Volume {LBAP-M01} to add: _____ mL
- 3) Add calculated amount of {LBAP-M01} to bottle containing reagent. Vortex till reagent is dissolved

LBAP-ADD05: 1M CaCl_2

- 1) Weigh out at least 1.11 g CaCl_2 for 10 mL 1M CaCl_2 and pour into a 15 mL conical vial {LBAP-M15}
- 2) Calculate HPLC-grade water {LBAP-M01} to add by dividing amount weighed by 110.98 mg
 - Amount weighed: _____ mg
 - Divide by: 110.98 mg/mL
 - Volume {LBAP-M01} to add: _____ mL
- 3) Add calculated amount of {LBAP-M01} to bottle containing reagent. Vortex till reagent is dissolved

LBAP-ADD06: 2.27M Sodium Butyrate

- 1) Resuspend 1g bottle in 4 mL HPLC-grade Water {LBAP-M01}.
- 2) Create 440 uL aliquots. Store at -20°C in additives box.

LBAP-ADD07: 250mM AEBSF

- 1) Resuspend 500mg bottle in 8.344 mL HPLC-grade Water {LBAP-M01}
- 2) Create 500 uL aliquots. Store at -20°C in additives box. Stable for up to 6 months

LBAP-ADD08: 5uM Microcystin LR

- 1) Resuspend 500 ug bottle in 1.005 mL DMSO {LBAP-M02}. Stock Bottle is now at 500 uM.
 - a) Slowly pipette DMSO up and down till you can see all flakes dissolved. You can also invert up and down to mix.
- 2) To make 5 uM LR Microcystin stocks, add 30 uL of 500 uM stock bottle to 2970 uL DMSO{LBAP-M02} in a 3 mL tube. Invert 7 times to mix.
- 3) Create 200 uL aliquots. Store 500 uM stock bottle and 5uM aliquots at -20°C in additives box.

LBAP-ADD09: 10% NP40

- 1) Add 180 mL HPLC-Grade Water {LBAP-M01} to pre-conditioned 250mL glass bottle {LBAP-M14}
- 2) Add 20 mL NP40 {LBAP-M12} to bottle
- 3) Invert 7 times to mix

LBAP-BUF01: Nucleus Buffer

- 1) Acquire pre-conditioned 500 mL glass graduated cylinder {LBAP-M15}
- 2) Add 42.79 g Sucrose {LBAP-M03} to cylinder
- 3) Bring up Sucrose to 250 mL with HPLC-grade water {LBAP-M01}
- 4) Add following amounts of Reagent Mixes {LBAP-ADD01-05}:
 - 30mL 1M KCl {LBAP-ADD01}
 - 7.5 mL 1M NaCl {LBAP-ADD02}
 - 7.5 mL 1M Tris pH 7.5 {LBAP-ADD03}
 - 25 mL 0.1M MgCl_2 {LBAP-ADD04}
 - 500 uL 1M CaCl_2 {LBAP-ADD05}
- 5) Add magnetic stir bar {LBAP-M17} (cleaned) to cylinder, stir till sucrose is dissolved

- 6) Remove magnetic stir bar. Bring up solution to 500 mL with HPLC-grade water {LBAP-M01}
- 7) Add solution to 500 mL filtration unit {LBAP-M18}, filter with vacuum
- 8) Remove filter and replace with the provided cap. Label, date, and initial bottle. Right contents on bottle
- 9) Store long term at 4°C