Institut für HIV Forschung
SOP#11-00 **(Aug 2017 - BS)**

SDS-PAGE GEL

Reagent	Manufacturer	Order Number
5M NaCl Solution	Sigma	S5150
Dithiothreitol (DTT)	Sigma	10197777001
HEPES	Sigma	H0887-100ml
Sodium dodecyl sulphate (SDS)	Applichem	A2572,0500
Roche Protease Inhibtor Cocktail	Sigma	04693132001
Tris-base	Sigma	T1503-1KG
EDTA pH 8.0	Sigma	03690-100ML
EGTA	Sigma	E3889-10G
Sodium orthovanadate	Sigma	S6508-10G
NP40	Abcam	ab142227
Glycerol	Sigma	G5516-500ML
Roti-Load 1 (reducing reagent)	Carl Roth	K929.2
Glycine	Roth	3790.2
Methanol	Roth	X948.1
10X SDS Running Buffer	Bio-Rad	1610732
4–20% Mini-PROTEAN®TGX™ Precast Gel	Bio-Rad	4561093EDU
Cell scraper	TPP	99003
PBS	Life Technologies	14190-144
Protein Standard Ladder	Bio-Rad	161-0374

Lysis Buffers:

There are several different lysis buffers (NP-40, Tris-HCl). Each has their own advantages/disadvantages. The one described below is best for whole cell extracts and membrane bound proteins

Cell Lysis Buffer (100mL):

Reagent	Stock Concentration	Volume of Stock	Final Concentration
NaCl Solution	5M	400µl	20mM
HEPES	1M	5mL	50mM
DTT	1M	100µl	1mM
EDTA pH 8.0	500mM	200µl	1mM
NP40	10%	5mL	0.5%
Glycerol	100%	10mL	10%
Sodium orthovanadate	0.5M	200µl	1mM
EGTA	250mM	400µl	1mM
Roche Protease Inhibtor Cocktail	-	1 tablet	
dH_2O	-	Up to 100mL	-

Store solution at 4°C for up to 2 months.

*Dissolve 5g of Sodium Deoxycholate in 50mL dH2O to make a 10% stock solution. The solution is stable at room temperature but is sensitive to light so protect with aluminum foil.
#To make 1L stock 100mM Tris-HCl solution, dissolve 121.14g Tris-HCl in 800mL dH2O. Calibrate the pH meter and use 10M NaOH to bring the pH up to 8.0. After pH, bring up to 1000mL with dH2O.
NOTE: Protease inhibitors will be added to lysis buffers just prior to adding to cells

SDS-PAGE Buffers:

Reducing Reagent:
We use Roti-Load 1 solution as a reducing reagent for proteins derived from cell lysates. This solution is provided in a 4X concentrate and will be diluted to 1X using your protein lysate prior to sample boiling.

Running Buffer (1L):

****Use the 10X TGS Running Buffer from Bio-Rad on the shelf by pouring 100ml into a 1L graduated cylinder and bringing it up to 1000mL with dH_2O. If there isn't any 10X TGS Buffer left, use the recipe below to make homemade Running Buffer****

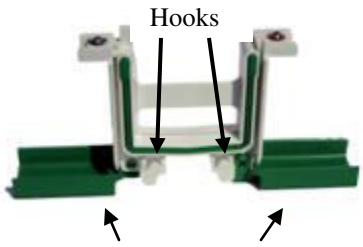
Reagent	Stock Concentration	Volume of Stock	Final Concentration
Tris base pH 8.0	250mM	100mL	25mM
Glycine	1.9M	100mL	190mM
SDS	10%	10mL	0.1%
dH2O	-	790mL	-

*Alternatively we keep a 10X solution of Tris base/Glycine on the shelf. Use 100mL of this 10X stock and add 10mL 10% SDS and 890mL dH2O.

Protocol:

1. Thaw Protease Inhibitors (labelled “PI” in -20C) on ice and add 100ul PI/mL of lysis buffer. Mix solution and keep on ice.
2. For suspension cells: transfer cells to FACS tube or 15mL conical and spin down at 350xg, 5min, 4°C. Aspirate supernatant off and add 5mL ice cold PBS to tube. Mix cells and spin again 350xg, 5min, 4°C. Aspirate PBS off cells and add 1mL/10^7 cells of ice cold lysis buffer. Mix thoroughly with a P1000 pipette. Transfer cell suspension to a 1.7mL Eppy and place on ice for 30min
    a. For adherent cells: Aspirate culture media and add 1-2mL of ice cold PBS. Move the plate in a small circular motion for ~10sec then aspirate PBS and add 1-2mL of ice cold lysis buffer. Scrape adherent cells using a cell scraper and transfer to a 1.7mL Eppy tube and place on ice for 30min.
3. Place Eppy tubes in a pre-cooled microcentrifuge (4°C) in the molecular lab and spin tubes at 12,000xg, 20min
4. Carefully remove Eppy tubes and place on ice
5. Transfer the supernatant of each tube to a new 1.7mL Eppy, taking care not to disturb the cell pellet
6. If desired, perform a protein quantification assay to determine protein content in each sample. (Pierce BCA Assay, use program “BCA micro_BS”)
    a. This is important when comparing multiple samples against one another. One should load the amount of protein in each in this case
7. SDS-PAGE gels are normally loaded with between 20-30ug of total protein per well (or 10-100ng purified protein). Choose a quantity (e.g. 25ug) and transfer volume equal to that mass to a new 1.7mL Eppy for each lysate (alternatively, use all of your sample)
8. Since the reducing agent is provided at 4X concentration, dilute to 1X by adding 1/3 Volume of Roti-Load 1 to each new aliquot of protein lysate (example: tube 1 has 12ul and tube 2 has 15ul, so tube 1 gets 4ul Roti-Load 1 and tube 2 gets 5ul.)
9. Boil each mixture in the heat block at 100C for 5-10min.
    a. Any remaining lysate can be frozen in the -20C for future use. Aliquot sample to avoid repeated freeze-thaw cycles.
10. While the sample is boiling, prepare the SDS-PAGE apparatus.

Tank + Lid



Green Side Arms

Electrode Assembly

    a. Remove the electrode assembly from the tank and fold the green side arms down

b. Retrieve a pre-cast gel from the 4C and open the package. Remove the green tape on the bottom
c. Place the pre-cast gel on the electrode assembly such that the numbers are facing correctly towards you. This means the gel cassette will have its shorter side facing towards the middle of the electrode assembly
    i. Push sure to push the gel cassette firmly against the electrode assembly and slide it down so that the bottom of the gel cassette is behind the hooks on the electrode assembly
d. If you require two gels, then repeat steps 9a-9c for the other side, otherwise use our empty gel cassette (you must always have 2 gel cassettes on the electrode assembly)
e. Squeeze both cassettes towards the middle and simultaneously fold the green side arms up to lock them in as in the right most part of the picture above.
f. Place the assembly inside the tank (make sure the colors on the electrode match the colors on the sides of the tank)
g. Fill the inside of the electrode assembly with Running Buffer until the buffer reaches the top of the gel cassettes
h. Fill the tank with Running Buffer up to the line indicated on the outside of the tank ("2 gels" or "4 gels")
i. Finally, remove the well comb from the gel cassettes. This is done when the gel is submerged in Running Buffer so that way the wells are not destroyed

11. After the sample is finished boiling, pipette mix each sample
12. Retrieve Protein Ladder from -20C.
13. Load 5ul into the first well on the SDS-PAGE gel. Note that you must pipette in the inner chamber of the electrode assembly to reach the well
14. Load 20-30ug of reduced protein (up to a max of 30ul) into each well
15. Place lid on the electrode assembly and tank, being sure the colors on the lid cables match the electrode colors (red to red and black to black).
16. Connect the lid cables to the power pak (match those colors!) and set the machine to run at a constant Voltage of 100V for 1-2hr
    a. You can check whether it is running by looking for bubbles forming on the wire inside the electrode assembly
    b. When you do not see a steady stream of bubbles all along the wire then check the how many milliAmps (mA) the system is at. The system should not go below 20mA during the run. If you have a system running at low mA (1-10mA) then the chamber is not sealed and running buffer is leaking from the inner chamber to the tank. Stop the run, adjust the gel cassettes, and refill the inner chamber by transferring running buffer from the tank back into the inner chamber using a pipettor.
    c. Let the gel run until the bottom marker of the protein ladder is almost to the bottom of the gel
17. After the run, turn off the power pak and remove the cables. Take off the lid and take the tank to the sink
18. Set the tank in the sink and remove the electrode assembly. Open the green side arms and retrieve your gel cassette
19. To access the gel, use a gel opener (green plastic tool) to pry open the sides of the gel cassette CAREFULLY.

    a. Opening the cassette too fast may lead to your gel ripping. If your gel will not fall to one side, re-wet the cassette in running buffer.
20. After the gel cassette has been opened, use the gel opener or the other side of the gel cassette to cut the wells off of the gel. Make a straight line directly under the well and press firmly to dislodge them
21. Now that the gel is out you can do 2 things:
    a. Western Blot (See SOP# 11-01)
        i. WB is uses antibodies to detect proteins among whole cell lysates.
    b. Coomassie/Silver staining (See SOP# 11-02)
        i. These staining techniques are used to visualize purified proteins
22. DO NOT STORE GEL OVERNIGHT. Some labs will wrap the gel in a wet paper towel and keep it overnight in the 4C. This will often lead to your protein diffusing throughout the gel and you will no longer have a sharp, crisp band