ANIMAL CELL TECHNOLOGY AND DOWNSTREAM PROCESSING GROUP

DEVELOPMENTAL OPERATING PROCEDURE

Title: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Adenovirus (Ad).	**Page** 1 **of** 16
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1.0 Objective

This procedure gives specific information in order to perform the SDS-PAGE of Ad samples.

2.0 Scope

The electrophoresis of Ad is useful to determine the purity and identity of the major protein components of the Ad under reduced conditions. The Silver Stain Plus (BIO-RAD) method is selected here because of its high sensitivity. The analysis of the gel is done by visual inspection only, comparing with a CsCl purified Ad standard included in the gel.

3.0 Responsibilities

3.1 It shall be the responsibility of the supervisor to:

3.1.1 Ensure that this procedure is followed by all Animal Cell Technology and Downstream Processing personnel performing the Ad purity and identity analyses on SDS-PAGE.

3.1.2 Ensure that the personnel working on the analyses are trained to work in Biosafety Level 2 Laboratory (BSL-2).

3.1.3 Ensure that the security rules are applied at the Biotechnology Research Institute (BRI) concerning the shipping and receiving of biorisk-2 agents.

4.0 Material

Material	Supplier	Catalogue #
Low Molecular Weight standard	Pharmacia	17-0446-01
Prestained Low Molecular Weight standard	BIO-RAD	161-0305
2-Mercaptoethanol	BIO-RAD	161-0710
Ready Gel 4-15% Tris-HCl	BIO-RAD	161-1104
Silver Stain Plus Kit	BIO-RAD	161-0449
Cellophane sheets	Novex	Large NC2200

5.0 Equipment

Mini PROTEAN® II system (BIO-RAD) Cat # 165-3375

6.0 Procedure

6.1 Samples

6.1.1 The preparation of Ad samples is done in the BSL-2 Laboratory using a biological safety cabinet.

6.1.2 The quantity of proteins in the sample is estimated by the Bradford method (BIO-RAD protein assay).

6.1.3 The 5× concentrated running buffer has to be diluted 1/5 with cold Milli-Q H_2O.

6.1.4 The samples must be diluted first in 1× running buffer then reduced with the reducing sample buffer (2 parts of sample + 1 part of reducing sample buffer) to get the final concentration as per the

following description:

For the silver stained and Western gels, an amount of 450 ng of protein should be loaded per well. Fill the batch records of the SDS-PAGE in the Annex 2.

The samples are heated at 100°C for 5 minutes.

The samples are centrifuged at 16,000 ×g (12000 RPM) for 2 minutes using an Eppendorf benchtop centrifuge.

Load 20 µL of each sample to the corresponding wells. It is recommended to run the electrophoresis of two gels simultaneously in order to compare the silver stained gel with the Western blot.

6.2 Molecular Weight Standards

6.2.1 Silver Stain: the low molecular weight standards (Pharmacia cat # 17-0446-01) is first diluted 1/85 in 1× running buffer then diluted 2:1 in reduced sample buffer and 20 µL is loaded.

6.2.2 Western blot: load directly 10 µL of a prestained standard (BIO-RAD low molecular weight standard cat # 161-0305).

6.3 Running conditions

The gels must be run for 10 minutes at 70 Volts and for 60 minutes at 140 Volts when using BIO-RAD Ready Gels 4-15% Tris-HCl.

6.4 Silver staining

Follow the instructions of the Silver Stain Plus Kit from BIO-RAD (Cat # 161-0449).

To prevent silver deposits on staining trays and inconsistent staining, all containers used for mixing and staining should be scrupulously cleaned with Sparkleen, rinsed with Milli-Q H_2O, cleaned with 50% Nitric Acid (10 minutes), and again rinsed thoroughly with Milli-Q H_2O, prior to use.

Fill the batch records of the Silver Stain Plus (BIO-RAD) in the Annex 3.

Fixative Step:
After gel electrophoresis, place the gel dedicated for the silver stain in the Fixative Enhancer Solution. With gentle agitation fix the gel for a minimum of 20 minutes.

Rinse Step:
Rinse the gel 2×10 minutes with Milli-Q H_2O (with gentle agitation).

Staining and Developing Step:
Develop the gel for approximately 20 minutes or until desired staining intensity is reached.

Stop Step:
Discard the development solution in the appropriate waste container and stop the staining reaction with 5% acetic acid solution for a minimum of 15 minutes.

6.5 Preparation for storage:
Place the gel in 5% Glycerol, 10% Acetic Acid for a minimum of 30 minutes.

6.6 Gel Scanning:
Rinse the gel with Milli-Q H_2O to remove the acetic acid. The gel is ready to be scanned and must be saved in a .tif format (for example), in order to have an electronic copy of the SDS-PAGE.

6.7 Gel Drying:
After having rinsed the gel with Milli-Q H_2O (3×10 minutes) place the gel between two cellophane sheets from Novex and let it dry at room temperature.

6.8 Solutions Preparation

See annex 1 for the batch records of the preparation of solutions.

6.9 SDS-PAGE

See annex 2 for the batch records of the SDS-PAGE.

6.10 Silver staining

See annex 3 for the batch records of the Silver Stain Plus (BIO-RAD)

7.0 Acceptance Criteria

7.1 The analysis of the reduced SDS-PAGE (Silver Stained) of Ad is done by visual inspection only. The Ad sample is compared with a CsCl purified Ad standard included in the gel.

7.2 In our case, we want to purify Ad using chromatographic steps (scalable process) in order to reach the same purity as the CsCl purified Ad std (not scalable process). The protein band profile of the Ad sample in the purification process, is checked visually and compared with the protein band profile of the CsCl purified Ad std.

8.0 Results

9.0 References

9.1 MINI-PROTEAN®II DUAL SLAB CELL (BIO-RAD),Instruction manual.

9.2 Silver Stain Plus Kit (BIO-RAD), Instruction manual.

ANNEX I

PREPARATION OF SOLUTIONS

(Electrophoretic Grade Reagents)

5´ Concentrated Running Buffer pH 8.3 1000 mL

Add 800 mL of Milli-Q H_2O to a beaker.
Weigh 15 g of Tris and add this component to the Milli-Q H_2O.
Weigh 72 g of Glycine and dissolve in Milli-Q H_2O.
Weigh 5 g of SDS and add this component to the Milli-Q H_2O.
Complete to 1000 mL with Milli-Q H_2O using a 1000 mL volumetric flask.
Check the pH, do not adjust it.
Store at 4°C.

Lot # (Preparation date DD-MM-YY):_____ Expiry date:_____

Desired volume:_____mL Final volume:_____mL

Balance #_____

Amount of Tris weighed:_____g Supplier:_____
Lot #_____

Amount of Glycine weighed:_____g Supplier:_____
Lot #_____

Amount of SDS weighed:_____g Supplier:_____
Lot #_____

pH check:_____at _____°C

Stored at:_____°C

Prepared by:_____Date:_____

ANNEX 1 continued

SDS-PAGE Sample buffer (BIO-RAD concentrated) (Sample 2:1 in sample buffer)

(20 mL when reduced after addition of β-mercaptoethanol).

(Electrophoretic Grade Reagent)

Use a 25 mL glass cylinder.
Add 6 mL of 0.5 M Tris-HCl, pH 6.8 (final 150 mM reduced).
Add 5 mL of glycerol (final 25% reduced).
Add 6 mL of 20% (w/v) SDS (final 6% reduced).
Add 0.6 mL of 4% Bromophenol Blue (final 0.12% reduced).
The final volume of this non-reduced solution is 17.6 mL.
This solution can be aliquoted in 250 µL and frozen at –20°C.
This solution can be kept several months at –20°C.
To obtain a reduced solution add 30 µL of β-mercaptoethanol to an aliquot of 250 µL immediately before use.
Samples should be diluted 2:1 (2/3 dilution) with sample buffer before loading on the gel. When the reduced sample buffer is with the sample, we obtain Tris-HCl 49.5 mM, Glycerol 8.25%, SDS 1.98%, 2-Mercaptoethanol 3.54%, Bromophenol Blue 0.04%. When using the SDS-PAGE sample buffer, heat the diluted sample for 5 minutes at 100^0C prior to loading.

LOT # (preparation date DD-MM-YY):_____
Desired volume:_____mL Final volume:_____mL

0.5 M Tris-HCl, pH 6.8	Volume:_____mL Lot #_____
20% (w/v) SDS	Volume:_____mL Lot #_____
Glycerol	Volume:_____mL Supplier:_____ Lot #_____
4% Bromophenol Blue	Volume:_____mL Lot #_____

Prepared by:_____Date:_____

ANNEX 1 continued

0.5 M Tris HCl pH 6.8 100 mL

Add 80 mL of Milli-Q H_2O to a beaker.
Weigh 6.057 g of Tris (Electrophoretic Grade)
Add this component to Milli-Q H_2O.
When it is completely dissolved, adjust the pH to 6.8 with 1N HCl.
Complete to 100 mL with Milli-Q H_2O using a 100 mL volumetric flask.
Filter through a 0.45 µm HA Millipore filter by vacuum.
This solution can be stored at 4°C for several months.

LOT # (Preparation date DD-MM-YY):_____Expiry date:_____

Desired volume:_____mL Final volume:_____mL

Balance #_____

Amount of Tris weighed:_____g Supplier:_____
Lot #_____

Initial pH:_____ Adjust the pH to 6.8 with 1N HCl.
Volume used to adjust the pH:_____mL (~ 3.5 mL)
Acid Lot #_____
Final pH:_____
Stored at:_____ ^{o}C

Prepared by:_____Date:_____

ANNEX 1 continued

20% Sodium Dodecyl Sulfate (SDS) 20 mL

Add 10 mL of Milli-Q H_2O to a beaker.
Weigh 4 g of SDS (Electrophoretic Grade)
Add this component to the Milli-Q H_2O, when it is completely dissolved complete to 20 mL with Milli-Q H_2O using a graduated cylinder. It may be necessary to slightly heat the solution in order to dissolve the SDS. Let cool to room temperature before completing to 20 mL with Milli-Q H_2O.
This solution can be stored at room temperature for several months.

LOT # (Preparation date DD-MM-YY):_____ Expiry date:_____

Desired volume:_____mL Final volume:_____mL

Balance #_____

Amount of SDS weighed:_____g Supplier:_____
Lot #_____

Stored at:_____^{o}C

Prepared by:_____ Date:_____

ANNEX 1 continued

4% Bromophenol Blue 10 mL

Add 8 mL of Milli-Q H_2O to a beaker.
Weigh 0.4 g of Bromophenol Blue.
Add this component to the Milli-Q H_2O, when it is completely dissolved complete to 10 mL with Milli-Q H_2O using a graduated cylinder.
Filter over a Whatman #1 filter paper.
This solution can be stored at room temperature for several months.

LOT # (Preparation date DD-MM-YY):_____Expiry date:_____

Desired volume:_____mL Final volume:_____mL

Balance #_____

Amount of Bromophenol Blue weighed:_____g
Supplier:_____
Lot #_____

Filtration Whatman # 1 Lot #_____

Stored at:_____^{o}C

Prepared by:_____ Date:_____

ANNEX 1 continued

5% Acetic Acid in Milli-Q H_2O 1000 mL

Add 900 mL of Milli-Q H_2O into a graduated beaker.
Add 50 mL of Acetic Acid.
Complete to 1000 mL with Milli-Q H_2O using a volumetric flask.
Store at room temperature.
This solution is stable for several months.

Lot # (Preparation date DD-MM-YY):_____Expiry date:_____

Desired volume:_____mL Final volume:_____mL

Acetic Acid:_____mL Supplier:_____
Lot #_____

Prepared by:_____ Date:_____

ANNEX 1 continued

5% Glycerol, 10% Acetic Acid in Milli-Q H_2O 1000 mL

Add 800 mL of Milli-Q H_2O into a graduated beaker.
Add 100 mL of acetic acid.
Add 50 mL of glycerol. Stir well.
Complete to 1000 mL with Milli-Q H_2O using a volumetric flask.
Store at room temperature.
This solution is stable for several months.

LOT # (preparation date DD-MM-YY):_____Expiry date:_____

Desired volume:_____mL Final volume:_____mL

Glycerol:_____mL Supplier:_____
Lot #_____

Acetic acid:_____mL Supplier:_____
Lot #_____

Prepared by:_____ Date:_____

ANNEX 2

ELECTROPHORESIS #_____

Samples from_____

Date of electrophoresis_____

Gel description:_____

Gel Lot #_____Serial #_____Exp. Date_____

Sample Buffer: SDS-PAGE BIO-RAD concentrated lot #_____

Running Buffer 5× Tris/Glycine/SDS lot #_____Dilution 1/5 ❑_____

PREPARATION OF SAMPLES

Reduced samples: β-mercaptoethanol added to sample buffer .
Amount of β-mercaptoethanol _____µL lot # _____added to ____µL sample buffer
All samples diluted 2:1 in sample buffer ❑
Reduced samples place in boiling water for _____ minutes
All samples are centrifuged at 16,000 ×g in benchtop centrifuge for_____ minutes

# SAMPLE	DILUTION IN_____	SAMPLE VOLUME	SAMPLE BUFFER	ng LOADED✳

✳_____µL / well

Comments: _____

Prepared by:_____

ANNEX 2 continued

ELECTROPHORESIS #_____

Samples from_____

Date of electrophoresis_____

Gel description:_____

Gel Lot#_____Serial #_____Exp. Date_____

Sample Buffer: SDS-PAGE BIO-RAD concentrated lot #_____

Running Buffer 5× Tris/Glycine/SDS lot #_____Dilution 1/5 ❑_____

PREPARATION OF SAMPLES

Reduced samples: β-mercaptoethanol added to sample buffer .

Amount of β-mercaptoethanol _____µL lot # ____added to __µL sample buffer

All samples diluted 2:1 in sample buffer ❑

Reduced samples place in boiling water for _____ minutes.

All samples are centrifuged 16,000 ×g in benchtop centrifuge _____ minutes.

# SAMPLE	DILUTION IN_____	SAMPLE VOLUME	SAMPLE BUFFER	ng LOADED✳
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
____	_____	_____	_____	_____
_____	__	___	___	__
___				

✳_____µL / well

Comments: _____

Prepared by:_____

ANNEX 2 continued

Electrophoresis information

ELECTROPHORESIS #_____

Samples from_____

Date of electrophoresis_____

ELECTROPHORESIS CONDITIONS

Apparatus_____Power supply:_____

Buffer: _____Lot #_____

Start Time:_____ Volts: 70 V_____mA_____

Volts: 140 V:_____mA_____

End time_____ Volts 140 V:_____mA_____

STAINING Gel # 1 type_____Gel # 2 type_____

ANNEX 3

Silver Stain Plus (BIO-RAD)

Date of staining:_____

Gels:_____

Containers cleaned with: Sparkleen ❑ 50% Nitric Acid ❑

Fixative Enhancer Solution

Volume needed: 100 mL ❑

Methanol: 50 mL ❑ Supplier:_____ Lot #_____

Acetic Acid: 10 mL ❑ Supplier:_____ Lot #_____

Fixative Enhancer Concentrate: 10 mL ❑ Supplier:_____ Lot #_____

Milli-Q H_2O: 30 mL ❑

Staining and Developing

#1 Silver Complex Solution Lot #_____ Volume: 5 mL ❑

#2 Reduction Moderator Solution Lot #_____ Volume: 5 mL ❑

#3 Image Development Reagent Lot #_____ Volume: 5 mL ❑

Development Accelerator Solution Lot #_____ Volume: 50 mL ❑

Note: Prepare the Development Accelerator Solution Cat # 161-0448 (BIO-RAD) by dissolving 2.5 g of Development Accelerator Reagent (Control #_____) in 40 mL of Milli-Q H_2O. Complete to 50 mL with Milli-Q H_2O using a 50 mL volumetric flask.

STEPS

Fixative Step:_____min (minimum 20 minutes)

Rinse Step with Milli-Q H_2O: 10 min ❑

10 min ❑

Staining and Developing: _____min (approximately 20 minutes)

Stop Step with 5% Acetic Acid: _____(minimum 15 minutes)

Place in 5% Glycerol, 10% Acetic Acid solution:_____(minimum 30 minutes)