Single Gel system based in Ahn T et al (2001)

Luisa F. Jiménez Soto

Abstract

This protocol was standarized in our lab based on the publication of Ahn T et al (2001): **Polyacrylamide Gel Electrophoresis without a stacking Gel: Use of Amino Acids as Electrolytes. DOI:10.1006/abio.2001.5038**

These gels run similar to a gradient gel: In a 6% gel you will be able to separate proteins ranging from 270 kDa to 25 kD an dhave them in the same gel.

The adaptation to Stain-Free system was done based on the publication by Ladner CL et al (2004) : **Visible fluorescent detection of proteins in polyacrylamide gels without staining. DOI:10.1016/j.ab.2003.10.047**

We have applied this protocol in the following publications: *Zeitler AF, Gerrer KH, Haas R, Jiménez-Soto LF. Optimized semi-quantitative blot analysis in infection assays using the Stain-Free technology. J Microbiol Methods. 2016 Jul;126:38-41. doi: 10.1016/j.mimet.2016.04.016. PubMed PMID: 27150675.Jiménez-Soto LF, Haas R. The CagA toxin of Helicobacter pylori: abundant production but relatively low amount translocated. Sci Rep. 2016 Mar 17;6:23227. doi: 10.1038/srep23227. PubMed PMID: 26983895; PubMed Central PMCID: PMC4794710.Jiménez-Soto LF, Clausen S, Sprenger A, Ertl C, Haas R. Dynamics of the Cag-type IV secretion system of Helicobacter pylori as studied by bacterial co-infections. Cell Microbiol. 2013 Nov;15(11):1924-37. doi: 10.1111/cmi.12166. PubMed PMID: 23844976.*

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Before start

Pouring these gels is not much different than with other acrylamide gels:

- Read the safety information of ALL solutions before strating and take all measures necessary to protect yourself and those working around you.
- Clean your glass and combs throughly with alcohol to remove any dirt that may cause bubbles in your gel. If bubbles appear while pouring, tap gently the glass. They will tend to float (if you have luck!)
- If you add Trichlorethanol for the Stain-Free, make sure you solubilize the trichlorethanol properly BEFORE adding the TEMED solution (You will see how little white bubbles will disappear after throughly mixing the solution)
- We use 20-well combs, but in order to have well define bands, we use 1 mm thickness plates for this combs. 0.75 mm do not work well.

Protocol

Prepare your 2X buffer solution. Preferably the day before.

Step 1.

We prepare usually 250 ml of the buffer, then sterile filtrate it and make aliquotes of 50 ml.

Beware: Sometimes the solubilization of the aminoacids can take the whole day under mix conditions.

It has help to shorten the time when we set the pH as close as possible to the target pH and warm it up to 37°C - 40°C under mixing conditions.

Once the aminoacids solubilize, we measure pH again, set it right and fill to final volume.

DURATION
06:00:00

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You have to sterile filtrate with a 0,22 µm filter. Because of the Aminoacids, it is a perfect place for growth of fungi (mold).

Prepare your glass for casting

Step 2.

Mix the gel components

Step 3.

Here is the table we keep in the lab to mix components.

Solution	5 ml	10 ml	15 ml	20 ml	25 ml	30 ml	35 ml	40 ml	50 ml
5% gel									
Water	1,6	3,2	4,8	6,4	8,0	9,6	11,2	12,8	16,0
Bis-Acrylamide 30%	0,83	1,7	2,5	3,3	4,2	5,0	5,8	6,6	8,3
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,008	0,012	0,016	0,020	0,024	0,028	0,032	0,040	0,040
6% gel									
Water	1,3	2,6	3,9	5,2	6,5	7,8	9,1	10,4	13,0
Bis-Acrylamide 30%	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	10,0
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0

Ammonium Persulfate 10%	0,05	0,10	0,15	0,2	0,25	0,30	0,35	0,4	0,5
TEMED	0,008	0,012	0,016	0,020	0,024	0,028	0,032	0,040	0,040
7% gel									
Water	1,1	2,3	3,4	4,6	5,7	6,8	8,0	9,1	11,4
Bis-Acrylamide 30%	1,2	2,3	3,5	4,6	5,8	7,0	8,1	9,3	11,6
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,006	0,009	0,012	0,015	0,018	0,021	0,024	0,030	0,030
8% gel									
Water	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	10,0
Bis-Acrylamide 30%	1,3	2,7	4,0	5,3	6,7	8,0	9,3	10,6	13,3
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,006	0,009	0,012	0,015	0,018	0,021	0,024	0,030	0,030
9% gel									
Water	0,9	1,8	2,7	3,6	4,5	5,3	6,2	7,1	8,9
Bis-Acrylamide 30%	1,5	3,0	4,5	6,0	7,5	9,0	10,5	12,0	15,0
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,004	0,006	0,008	0,010	0,012	0,014	0,016	0,020	0,020
10% gel									
Water	0,6	1,3	1,9	2,6	3,2	3,9	4,5	5,2	6,5
Bis-Acrylamide 30%	1,7	3,3	5,0	6,6	8,3	10,0	11,6	13,3	16,6
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,004	0,006	0,008	0,010	0,012	0,014	0,016	0,020	0,020
13,6% gel									
Water	0,6	1,3	1,9	2,6	3,2	3,9	4,5	5,2	6,5
Bis-Acrylamide 40%	***1,7***	***3,3***	***5,0***	***6,6***	***8,3***	***10,0***	***11,6***	***13,3***	***16,6***
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,004	0,006	0,008	0,010	0,012	0,014	0,016	0,020	0,020
14,08% gel									
Water	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Bis-Acrylamide 40%	***2,3***	***4,7***	***7,0***	***9,4***	***11,7***	***14,1***	***16,4***	***18,8***	***23,5***
2X Single-gel buffer	2,6	5,2	7,8	10,4	13,0	15,6	18,2	20,8	26,0
Ammonium Persulfate 10%	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,50
TEMED	0,004	0,006	0,008	0,010	0,012	0,014	0,016	0,020	0,020

NOTES

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For Stain-Free gels, add Trichlorethanol solution 1:100

Pour the gel.

Step 4.

- Once TEMED is added, polymerization will start. While the solution stills liquid, pour it into the plate system (5 ml/mini-protean with space 0,75 mm) until the whole space is full.
- Taking care of not leaving bubbles, introduce the comb you need. Polymerization takes place in around 10-15 min

Run the gels.

Step 5.

Introduce the plate with the gel in a protein electrophoresis chamber , add the SDS- running buffer (for denaturing conditions) and run.

Since this gel system does not have a stacking gel, it is better if you run the first 10 min at 90 V so that the proteins enter slowly in the gel. Then increase the voltage to 140 V and run for 55min (6%) to 75 min(>10%), or 240V for 20 min.

Disclosure: The 240V for 20 min is only achieved using the Bio-Rad Running buffer.

NOTES

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I am almost sure that I could increase the voltage above 240V with the Bio-Rad buffer, but only if I run the gel in cooling conditions or in pre-cooled buffer. The heat production at V >240V is visible in the way the proteins run.

Warnings

Acrylamide is a neurotoxin. Avoid any contact with it. Apply all safety measures required to avoid contact or contamination of surfaces.