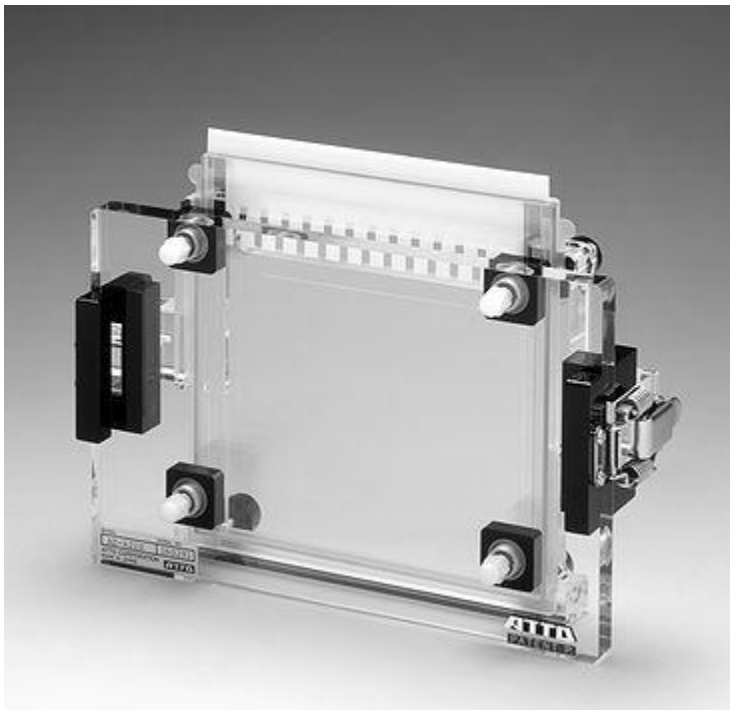
AE-6210 Slab Gel Cast



Operation Manual

June 2006
Version 2.0
ATTO Corporation

Safety Warning

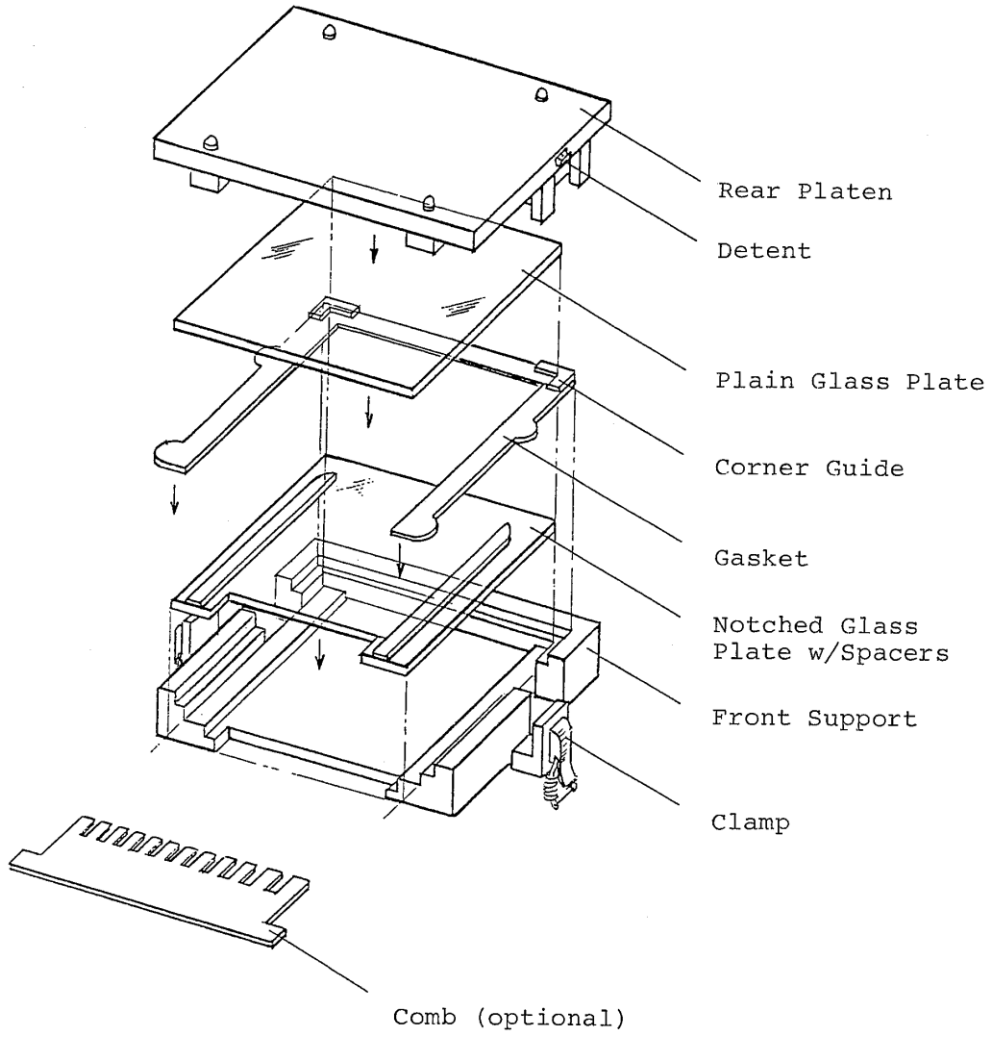
- Prior to using this apparatus, operating and/or handling instructions of this apparatus and apparatus, reagents, and/or chemicals that are used with this apparatus must have been understood.
- Use of this apparatus includes application of electricity, which may be fatal or injurious to human. Care should be taken against electricity when applying it to this apparatus.
- Use of this apparatus includes handling of reagents and/or chemicals that may be chemically hazardous or carcinogenic. Care should be taken for protection from hazardous or carcinogenic reagents and/or chemicals when handling them.
- Environments of high temperature, high humidity, dust, corrosive has, excessive source-voltage fluctuation excessive electrical noise, and physical shock or vibration should be avoided.
- This apparatus and apparatus that are used with this apparatus should be installed on a flat, level, solid and stable surface.
- Prior to operating this apparatus, the apparatus and its components and/or parts should be thoroughly clean and dry. Caution should be exercised against water or moisture which may cause electrical hazards.

1. SPECIFICATIONS

Application: Preparation of polyacrylamide slab gel
Gel size: 138 mm wide, 130 mm long
Plate size: 160 x 160 cm
Gel thickness: 1 or 2 mm
Number of sample wells: 8, 12 or 20
Materials:
Acrylic plastic, except stainless steel for clamps, glass for plates, silicone rubber for gasket and Teflon for combs.
Dimensions: 25 x 8 x 15 (W x D x H) cm
Weight: 1.1 kg

The gel prepared with this apparatus is accommodated by Atto AE-6200 and AE-6220 electrophoresis chamber.

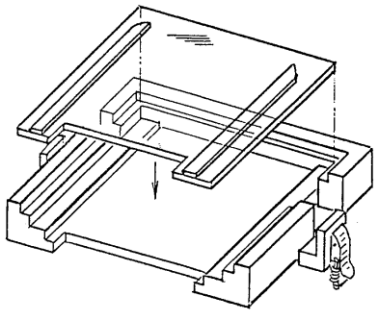
2. PART NAMES



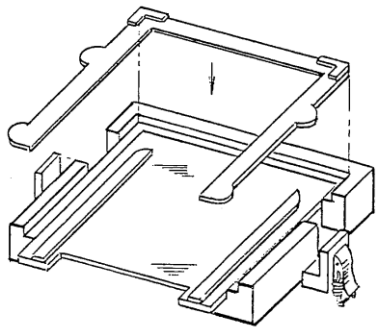
3. SET COMPOSITION(please refer to item which you ordered – 2392010 or 2392011)

Item	Quantity
2392010 AE-6210 Slab Gel Cast, 1 mm:	
Front support with two spring-clamps	1
Rear platen	1
Plain glass plate	1
Notched glass plate with 1 mm side-spacers	1
1 mm gasket	1
OR	
2392011 AE-6210 Slab Gel Cast, 2 mm:	
Front support with two spring-clamps	1
Rear platen	1
Plain glass plate	1
Notched glass plate with 2 mm side-spacers	1
2 mm gasket	1

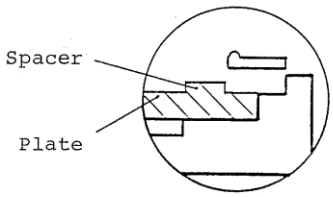
4. OPERATION

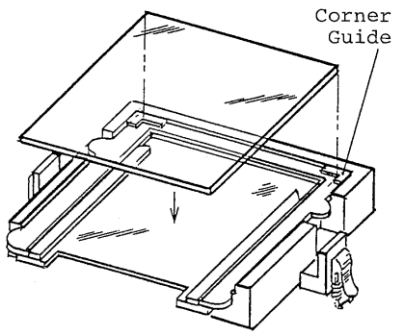


4.1
Lay the front support with its frames upper. Place the notched glass plate with its side-spacers upper in the front support.

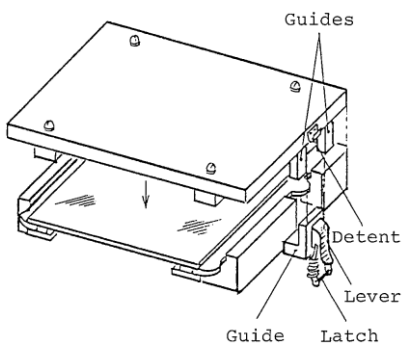


4.2
Place the gasket with its corner guides upper on the glass plate, along the side-spacers.

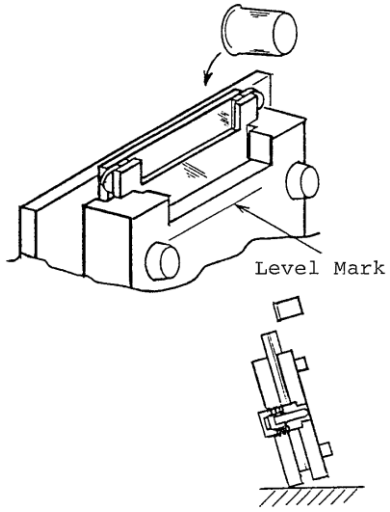




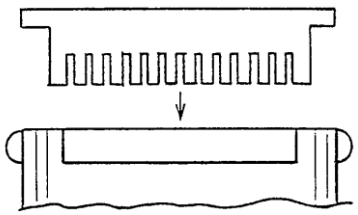
4.3
Overlay the plain glass plate. The two corners of the glass plate should be inside the corner guides of the gasket.



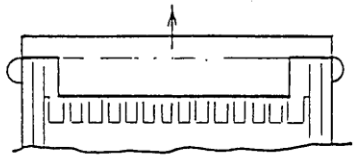
4.4
Overlay the rear platen, engaging the guides of the platen and the guides of the front support. Put the latches of the support on the detents of the platen, and lock them by lowering the levers of the support.



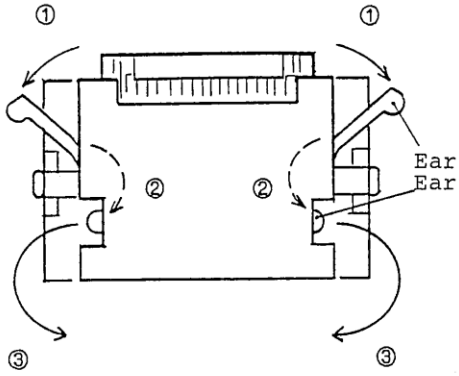
4.5
Stand the assembled gel cast. While slanting it, introduce separating gel solution into the gel cast up to the level mark on the front support. Overlay water to about 2 mm depth. Allow the gel solution to polymerize for about 1 hour.



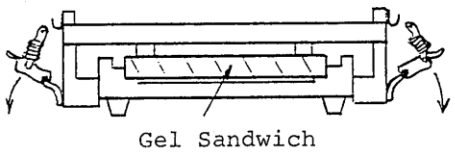
4.6
Discard the water. Rinse the top of the gel with a little volume of concentrating gel solution. Introduce concentrating gel solution. Immediately insert the well forming comb between the glass plates, taking care not to trap air bubbles. Allow the gel solution to polymerize for about 30 minutes.



4.7
Remove the comb, by pushing it up with fingers.



4.8
Remove the gasket, by firstly pulling the upper ears of the gasket and secondly pulling out the lower ears. Rinse the sample wells twice or three times with electrophoresis buffer with the use of a pipette.



4.9
Lay the gel cast with its front support lower. Release the clamps. Take out the gel sandwich.

5. APPLICATION TECHNIQUE

Note: There have been various techniques published for vertical electrophoresis with polyacrylamide gel. Here are described two techniques as a guide. One is the Laemmli's method with the utilization of SDS, which suits molecular weight determination of proteins. Another is a usual technique without utilizing SDS, which does not interfere with the 3-dimensional structures of proteins, i.e. does not denature proteins.

5.1 Laemmli's Method

5.1.1 Reagents:

Acylamide
N,N'-methylenebisacrylamide (Bis)
Ammonium persulfate
N,N,N',N'-tetramethylethylenediamine (TEMED)
Tris hydroxymethylaminomethane (Tris)
Sodium laurylsulfate (SDS)
Hydrochloric acid
Glycine
Glycerin
Acetic acid
Methanol
Coomassie Brilliant Blue G or R
2-mercaptoethanol
Bromophenol Blue (BPB)
Molecular weight marker, according to samples.

5.1.2 Stock Solutions

Solution A 30% acrylamide solution:
29.2 g Acrylamide
0.8 g Bis
Add water to make up 100 ml.

Solution B 1.5 M Tris buffer, pH 8.8:
Dissolve 18.17 g of Tris and 0.4 g of SDS in water, adjust for pH 8.8 with HCl, and make up to 100 ml.

Solution C 0.5 M Tris buffer, pH 6.8:
Dissolve 6.06 g of Tris and 0.4 g of SDS in water, adjust for pH 6.8 with HCl, and make up to 100 ml.

Solution D 10% ammonium persulfate:
Add 1 ml of water to 0.1 g of ammonium persulfate. Prepare just prior to use.

Note: The stock solutions A, B, and C can be stored for approximately 1 month in cold room.

5.1.3 Gel Solution for a 1 mm thick gel

Table 5.1.3 Composition: (quantity; in ml) (%; gel concentration)

	Separating gel					Concentrating gel	
	5%	7.5%	10%	12.5%	15%	20%	4.5%
Sol. A	3	4.5	6	7.5	9	12	0.9
Sol. B	4.5	4.5	4.5	4.5	4.5	4.5	-
Sol. C	-	-	-	-	-	-	1.5
Sol. D	0.07	0.07	0.07	0.07	0.07	0.07	0.018
TEMED	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water	10.5	9	7.5	6	4.5	1.5	3.6

Note: For a 2 mm thick gel, double the above quantities.

Mix Solution A, Solution B (or Solution C) and water, add TEMED and Solution D, and gently mix. Immediately cast a gel(s).

5.1.4 Stock Solution, 10% SDS solution

The stock solution is convenient for preparation of samples and buffer. Dissolve 10 g of SDS into water, to make up 100 ml.

5.1.5 Sample Preparation

Protein concentration in sample: 1 to 2 mg/ml.

Final concentration:	0.1 to 0.2%	Protein
	1%	SDS
	1%	2-mercaptoethanol
	10 mM	Tris-HCl buffer, pH 6.8
	20%	Glycerin

Heat: At 100°C for 1 to 2 minutes.

As an example of serum sample: (protein concentration in serum; 6 g/dl)

(1) Introduce 20 μl of serum into a test tube.
(2) Add 650 μl of water.
(3) Add 100 μl of 10% SDS solution.
(4) Add 10 μl of 2-mercaptoethanol.
(5) Add 20 μl of 0.5 M Tris-HCl buffer, pH 6.8 (Stock Solution C).
(6) Add 200 μl of glycerin.
(7) Seal the test tube with parafilm or such.
(8) Heat gradually in water, allow 1 to 2 minutes in boiled water, and take out.

5.1.6 Electrophoresis Buffer

Buffer:	0.025 M	Tris
	0.192 M	Glycine
	0.1%	SDS

Add 10 ml of 10% SDS solution to 3 g of Tris and 14.4 g of glycine, and make up to 1000 ml with water.

5.1.7 Marker Stain

Marker stain applied in sample wells prior to sample application provides ease of observing migration.

Marker stain:	1 mg	BPB
	100 μl	Glycerin
	900 μl	Water

5.1.8 Electricity

30mA constant current suits usual electrophoresis of serum samples, but current, voltage and time are to be evaluated according to samples and etc.

5.1.9 Staining & Destaining

Staining Solution:
Add water to 2.5 g of Coomassie Brilliant Blue, 500 ml of methanol and 100 ml of acetic acid, to make up 1000 ml.

Destaining solution:
Add water to 250 ml of methanol and 70 ml of acetic acid, to make up 1000 ml.

Easy preparation:

Stock solution:	1 volume	Acetic acid
	3 volumes	Methanol
	6 volumes	Water

Staining solution: Dissolve 0.25% volume of Coomassie Brilliant Blue in the stock solution.

Destaining solution: Use the stock solution as it is.

5.2 Usual Method without SDS

5.2.1 Reagents:

Same as 5.1.1, except sodium laurylsulfate (SDS), 2-mercaptoethanol and molecular weight marker which are not necessary.

5.2.2 Stock Solutions:

Same as 5.1.2, except Solution B and Solution C from which SDS is excluded.

5.2.3 Gel Solution:

Same as 5.1.2, except exclusion of SDS in the stock solutions.

5.2.4 Sample Preparation

Protein concentration in sample: 1 to 2 mg/ml.

Final concentration:	0.1 to 0.2%	Protein
	10 mM	Tris-HCl buffer, pH 6.8
	20%	Glycerin

As an example of serum sample: (protein concentration in serum; 6 g/dl)
(1) Introduce 20 μl of serum in a test tube.
(2) Add 760 μl of water.
(3) Add 20 μl of 0.5 M Tris-HCl buffer, pH 6.8 (Stock Solution C without SDS).
(4) Add 200 μl of glycerin.

5.2.5 Electrophoresis Buffer

Buffer:	0.025 M	Tris
	0.192 M	Glycine

Add water to 3 g of Tris and 14.4 g of glycine, to make up to 1000 ml.

5.2.6 Maker Stain

Marker stain applied in sample wells prior to sample application provides ease of observing migration.

Marker stain:	1 mg	BPB
	100 μl	Glycerin
	900 μl	Water

5.2.7 Electricity

30mA constant current suits usual electrophoresis of serum samples, but current, voltage and time are to be evaluated according to samples and etc.

5.2.8 Staining & Destaining

Staining Solution:
Add water to 2.5 g of Coomassie Brilliant Blue, 500 ml of methanol and 100 ml of acetic acid, to make up 1000 ml.

Destaining solution:
Add water to 250 ml of methanol and 70 ml of acetic acid, to make up 1000 ml.

5.3 References

Davis, B.T.: Disc-electrophoresis II. Method and application to human serum proteins, Annual N.Y. Acad. Sci., 121, 404 (1694).

Reid, M.S. and Bielski, R.L.: A Simple Apparatus for Vertical Flat-Sheet Polyacrylamide Gel Electrophoresis, Anal. Biochem., 22, 374-381 (1968).

Laemmli, U.K. Nature, 227, 680 (1970).

Howard, G.A. and Traut, R.R.: Separation and radiography of microgram quantities of ribosome proteins by two-dimensional polyacrylamide gel electrophoresis, FEBS Letters, 29, 177 (1973).

Studier, F.W.: Analysis of Bacteriophage T7 Early RNAs and Proteins on Slab Gels, J. Mol. Biol., 79, 237-248 (1973).

O'Farrell, P.H.: High Resolution Two-Dimensional Electrophoresis of Proteins, J. Biol. Chem., 250, 4407-4021 (1975).

6. MAINTENANCE

After usage, clean the apparatus and components with water and dry them at room temperature. Avoid organic solvents and high temperature which may damage them.

7. SPARE PARTS

Code No.	Name	
2392020	1 mm Plate Set, RS 1 pair of glass plates + 1 gasket, for 1 mm gel	
2392024	2 mm Plate Set, RS 1 pair of glass plates + 1 gasket, for 2 mm gel	
	Common for all gel thicknesses:	
2392031	Plain Glass Plates 2/pk, RS	Rectangular without notch & side-spacers
	Notched glass plates with side-spacers:	
2392029	1 mm Glass Plates 2/pk, RS	For 1 mm gel
2392030	2 mm Glass Plates 2/pk, RS	For 2 mm gel
	U-shaped opaque silicon rubber gasket:	
2392032	1 mm Gaskets 3/pk, RS	For 1 mm gel
2392033	2 mm Gaskets 3/pk, RS	For 2 mm gel
2392060	1 mm 8-well Comb, RS	
2392061	1 mm 12-well Comb, RS	
2392062	1 mm 20-well Comb, RS	
2392066	1 mm Blank Comb, RS	
2392078	1 mm Preparative Comb, RS	1 sample well + 1 reference well
2329735	1 mm 28-well Comb, RS	special order item
2392063	2 mm 8-well Comb, RS	
2392064	2 mm 12-well Comb, RS	
2392065	2 mm 20-well Comb, RS	
2392067	2 mm Blank Comb, RS	
2392076	2 mm Preparative Comb, RS	1 sample well + 1 reference well
2392013	Base Unit, AE-6210	Front support with clamps
2392014	Cover Unit, AE-6210	Rear platen
2392015	Spring Set, AE-6210	

Warranty

Atto Corporation warrants all its products subject to the terms and conditions set forth below.

1. This warranty covers all new products that are sold by Atto Corporation (hereinafter called Atto).

2. Expendable items are not covered by this agreement.

3. Claims under this warranty are limited to defects in material and workmanship of the products.

4. Malfunction and/or damage due to neglect, abuse, operation or repair contrary to specifications and/or instructions presented by Atto are not warranted.

5. Atto shall not be liable to consequential damage, labor, loss or expense directly or indirectly arising from us of the products.

6. Damage due to transit is not covered by this warranty.

7. The warranty period is one (1) calendar year from a date when the products are shipped from Atto to an original purchaser.

8. This warranty is not applied to any defect that is reported to Atto later than one (1) calendar month from a date of warranty termination.

9. Atto Shall supply parts to replace faulty parts of defective products under this warranty, free of charge.

10. Atto shall repair defective products under this warranty which cannot be repaired at field, free of charge.

11. Atto shall replace defective products under this warranty which cannot be repaired, free of charge.

12. Freight charges for return and replacement shipments under this warranty are shared by Atto and a purchaser, that is one way by either party and another way by another party.

13. Warranty period of repaired products and replacement products or parts is three (3) calendar months from a date when the said products or parts are shipped from Atto, or a remaining term of an original warranty period of the defective products, whichever lasts longer.

14. Return of the products for credit or refund is not accepted unless otherwise agreed in writing by Atto.

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