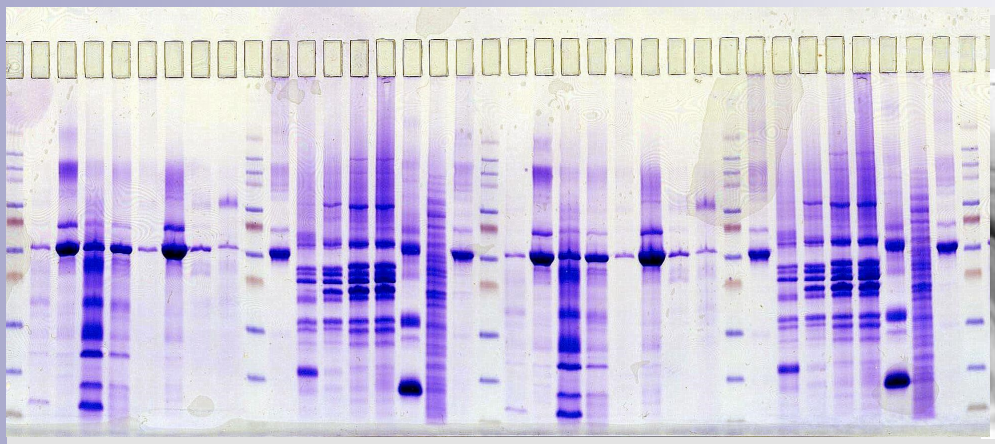
SDS-Electrophoresis with Urinary Proteins

V13 02/20



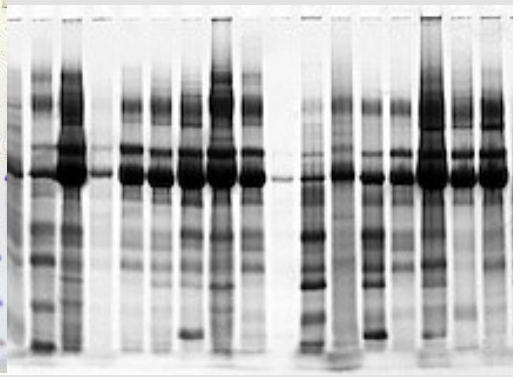


Fig. 2: *Prelabeling with UFO*

Fig. 1: *Urinary Protein Gel SDS Gradient. Stained with „Hot Coomassie". Run time 90 min.*

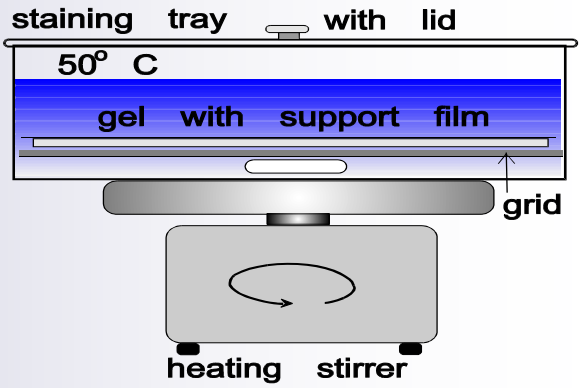
General: **Urinary Protein Gels are designed for horizontal electrophoresis. They run without buffer tanks, the electrode buffer is simply soaked into wicks and layed onto the edges of the gels. The gels can be cut adapted to the number of samples, this means: Low cost per electrophoretic lane!**
Using a new buffer system sharpest separation and long shelf life (1 year as Ready-To-Use gel) is achieved.
2 x 52S-gel (à 5 µl) can be run simultanously (bidirect) on one cooling-plate!
Especially for typing of the urinary proteins a sensitive and quantitative staining is necessary. Because urinary protein testing is a routine job the staining procedure should be as simple as possible.
Using the „Hot Coomassie Staining" we fulfill these demands. Figure 1.
Labeling the urinary proteins in the red channel using T-REX 310 gives another quantifyable method for routine application. Figure 2.

Hardware

flatbed IEF professional	edc-ief-2836
Staining Tray Normal	edc-wm-n1
DryPool Combi	edc-me-d

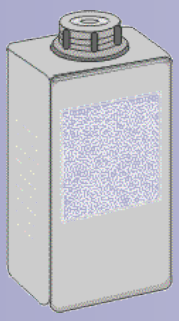
Consumables

Kit Urinary Proteins SDS 52S (à 5 µl) 4 gels, electrodes– and sample buffer 10X	edc-4011
Kit Urinary Proteins SDS gradient 4 gels, electrodes– and sample buffer 10X	edc-4010
Coomassie R350 tablets	GE 17-0518-01
Urinary protein labeling kit	Dyeagnostics „UFO"
Cooling Fluid	edc-2011



Performing „Hot Coomassie-staining"

The Sample Buffer „10x conc":



15 ml Sample Buffer 10X is supplied with this kit. To the samples only 10% (vol/vol) of this „10 x conc" buffer should be added. So the samples will be only slighly diluted. This sample buffer is non-reducing! Fluoresc. dyes can be added.

Hot Coomassie Staining-Procedure:

30 min 0.03% Coomassie in 12.5 % while stiring (50°-60°C, see picture above).

Destaining: 4h with 12.5 % HAc/10% EtOH.
Nicest destaining: 1 x overnight with 10% HAc
Preserving 20 min 5 % Glycerol.

See EDC's website: (—> staining —> dyestaining —> protdye—> hot Coomassie)

SDS-Electrophoresis with the 10X Sample Buffer

Sample Concentration

The protein concentration of the samples for a electrophoretic separation must be within a certain region. Taking too less means nothing can be detected afterwards - and too much of protein overloads the separation leading too unsharp bands.
The protein concentration range depends on the sensitivity of the visualization process following the electrophoretic procedure:

The Coomassie Staining:

This staining is less sensitive but can be quantificated.
Concentration range per slot with 13 µl sample volume: 3 µg (200 mg/l = 20 mg/dl)

The Silver Staining:

Very sensitive but no quantification possible.
Concentration per slot with 13 µl sample volume: 0.3 µg (20 mg/l = 2 mg/dl)

Fluorescence-Labeling: Using Dyeagnostics UFO the urinary proteins can be labeled before electrophoresis. For visualization a fluorescence-scanner is needed. Quantifyable! Prelabeling 30 min on ice after the SDS-treatment. Follow supplier's manual!

Urine Samples and the 10X Sample Buffer

The urines should be visualized with Coomassie because the validation of the results are qualitative and quantitative. The sample volume should be 13 µl (24S) or 5 µl (52S). Physiological urines show a characteristic low protein concentration. Only a weak Albumin-band should be detectable.
This is the reason a 10X sample buffer should be used: The urines will be diluted only by a 10% (v/v) instead of 50% (v/v) when a normal 2X sample buffer is applied.

Treatment of Urine samples

1.Dilution and SDS-Providing for Coomassie-staining, silver: 1:50, fluorescence*

Protein Concentration	Test - Stick	Urine	H_2O_{bidest}	10X Sample Buffer
negative		90 µl	—–	10 µl
0.3 g/l = 30 mg/dl		45 µl	45 µl	10 µl
1 g/l = 100 mg/dl		20 µl	70 µl	10 µl
5 g/l = 500 mg/dl		5 µl	85 µl	10 µl

*Fluorescence samples treatment and labeling: see the UFO manual from Dyeagnostics.

2. Do not reduce the Samples

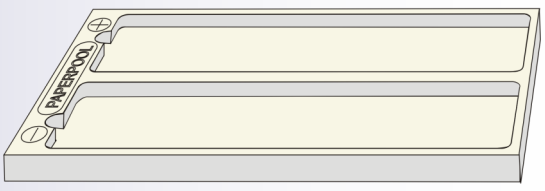
Reducing with mercaptoethanol or dithiothreitol will distroy the quaternary structures of the Immunoglobulins. These molecules show a native molecular weight of 160 kD, after reducing only heavy (43 kD) and light (20 kD) chains will appear.
Urinary proteins should run according their quarternary molecular weight. The separation distances of these not reduced SDS-protein-mycelles are not exactly relative to their molecular weights!To type the bands: Run known serum-protein samples (Albumin, IGG, ...) to compare to the urinary proteins.

3. Heating up to 95°C for 3 minutes

To coat the proteins with the SDS-molecules the sample-SDS-mixtures from above should be heated to 95°C for 3 minutes. The Eppendorf-cups are placed in an Eppendorf -Cup heater. After 3 minutes the SDS-protein mycelles are formed. This procedure leads to a uniform negative charging of the proteins and the molecular weights can be read out after the electrophoresis run. See the figure at the right side.

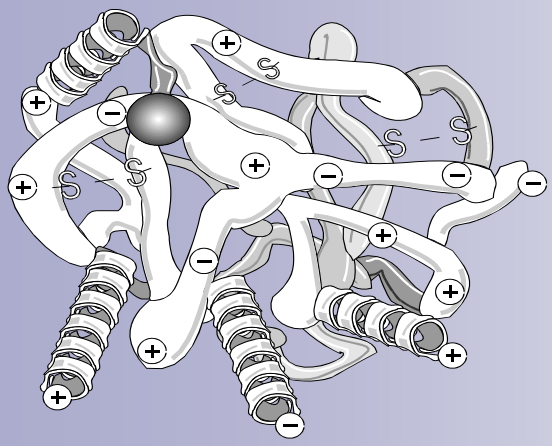
4. Electrophoresis and staining

Perform the electrophoresis and the staining according to the SDS-DryGel manual. Pictures of the main handling-steps on page 4.
Electrode buffer volume = 10 ml. See picture right.

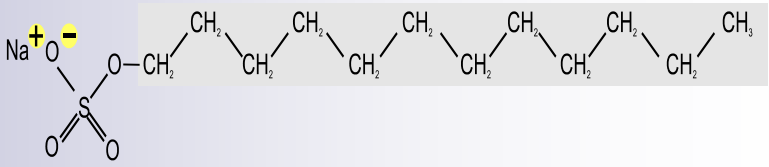


5. The mechanisme of SDS-treatment

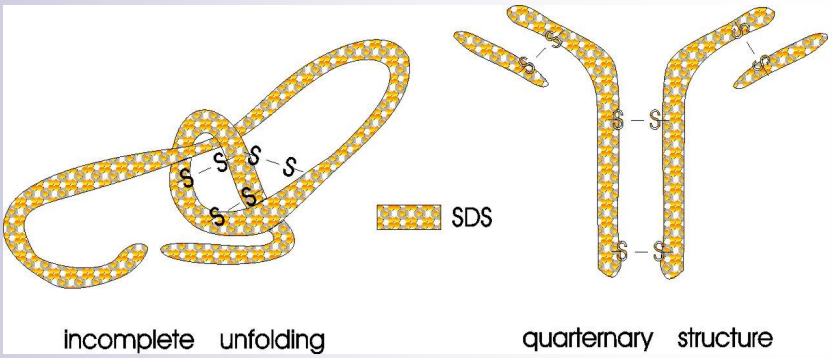
By heating up the proteins to 95°C for 3 minutes the proteins and the SDS-molecules will form SDS-protein mycelles. The original charge of the proteins will be covered by the negative charges of then tenside: These mycelles run according to their molecular weights.
If no reducing agent is present (Mercaptoethano or Dithiothreitol) the tertiary structure will not be destroyed, f.e. the IgG molecule stays at 160 kD instead of falling apart to the low and heavy chain.
Due to not completely unfolded molecules (intra-molecular disulfide-bridges) the relation of migration speed and the mol-weight of the proteins is not absolute correct.



The native protein



The Sodiumdodecylsulfate-molecule (SDS)



The SDS-protein mycelles, not reduced

6. Running Conditions (15°C)
(total ~1.5 hours)

Bio-Rad Power Pac HV: *type in this parameter as constant —>* n mA
(Select „BASIC" mode) *type in the other settings as limits —>* n V

Normal electrophoresis

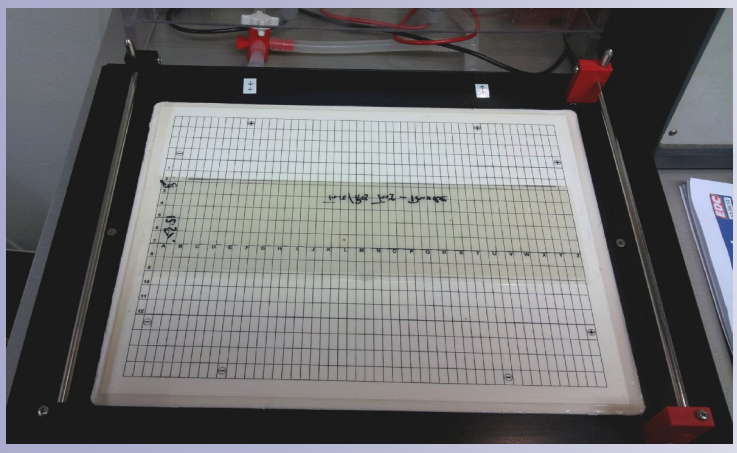
1 Gel:	Set V	Start Value	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	300 V	*(170 V)*	30 mA	10 W	25 min	buffer discontinuity through the slot
phase 2 (sample concentration)	500 V	*(280 V)*	42 mA	20 W	20 min	buffer discontinuity in the stacking gel
phase 3 (main electrophoresis)	800 V	*(460 V)*	60 mA	50 W	40 min	buffer discontinuity in the resolving gel

Bidirectional electrophoresis

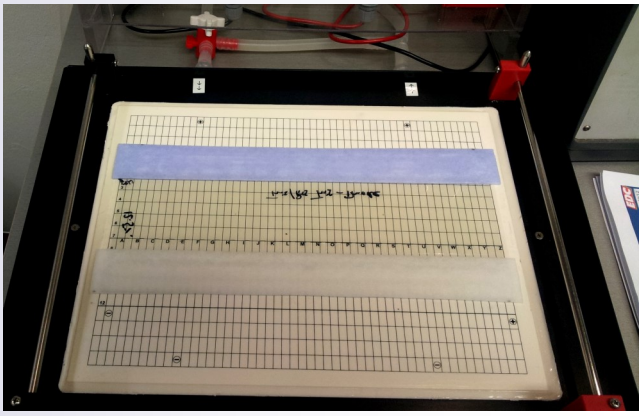
1 Gel:	Set V	Start Value	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	300 V	*(140 V)*	60 mA	20 W	25 min	buffer discontinuity through the slat
phase 2 (sample concentration)	500 V	*(250 V)*	85 mA	30 W	20 min	buffer discontinuity in the stacking gel
phase 3 (main electrophoresis)	650 V	*(400 V)*	100 mA	60 W	40 min	buffer discontinuity in the resolving gel

6. Handling of the UrinaryProtein-Gel (use the Cooling Fluid instead of Kerosene)

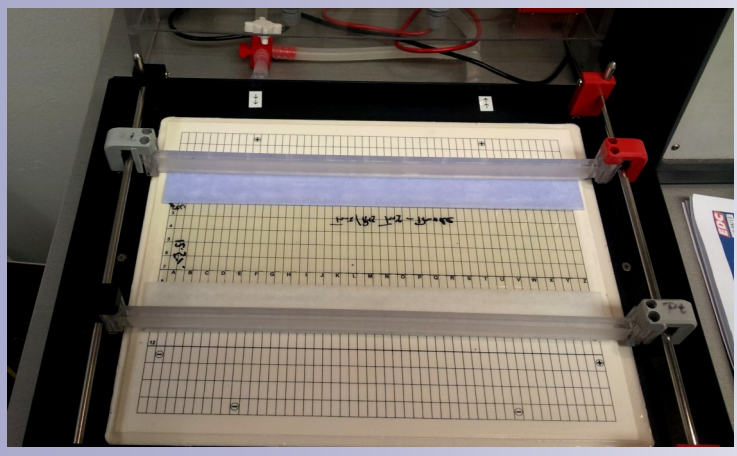
a) 1 x 24S gel per cooling plate. Only touch the gel at its side with the slots!



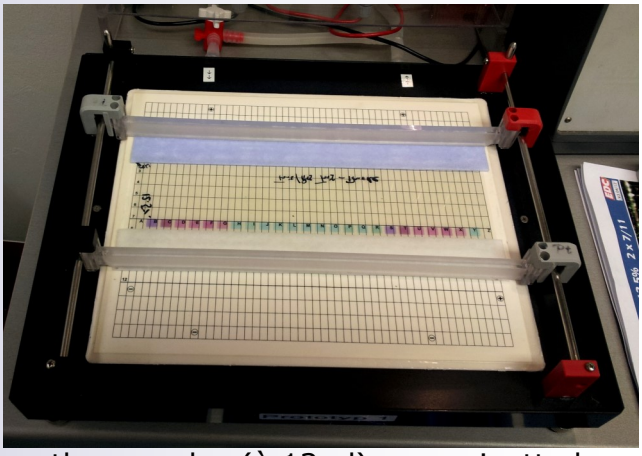
gel is placed on the cooling plate with the Cooling Fluid



the electrode-wicks (convex side to the top) overlap the gel ~2 mm



electrodes are placed at the wick's edges



the samples (à 13 µl) were pipetted

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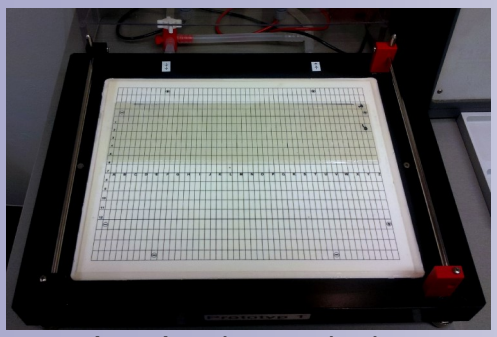
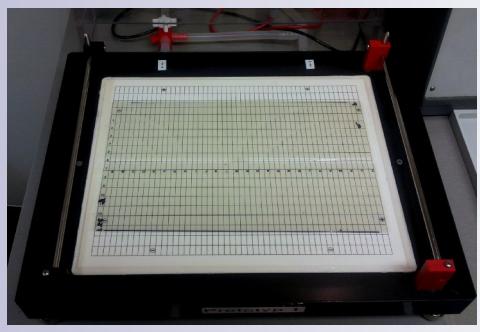
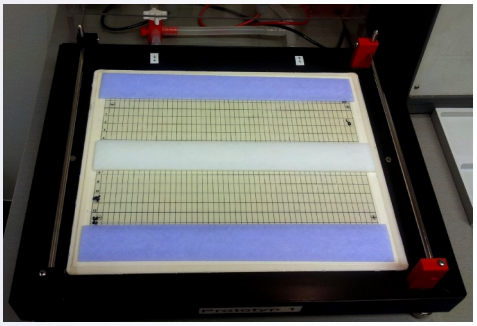
b) 2 x 52S gels running bidirect on one cooling plate



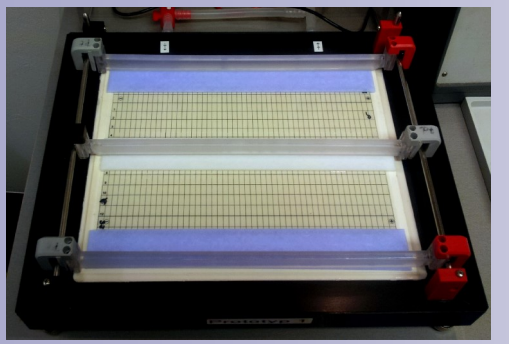
First (rear) gel is applied:
with its cathodal slot-edges to the line #4.



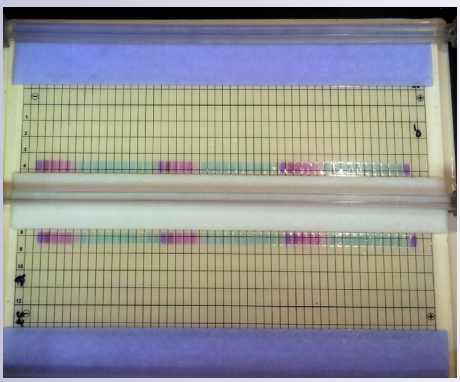
Second (front) gel is applied:
with its cathodal slot-edges to the line #8.



The double central cathode strips + the peripheral anodes strips are applied.
(convex side to the top)



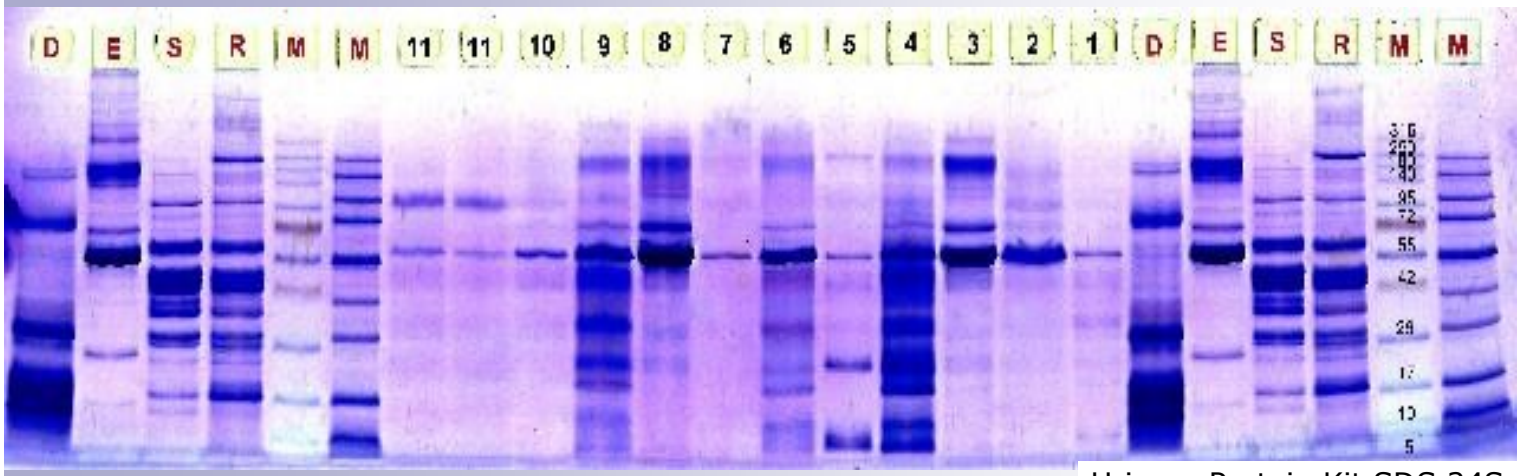
Electrodes sit on their strips



2 x 52 samples are filled (5 µl)

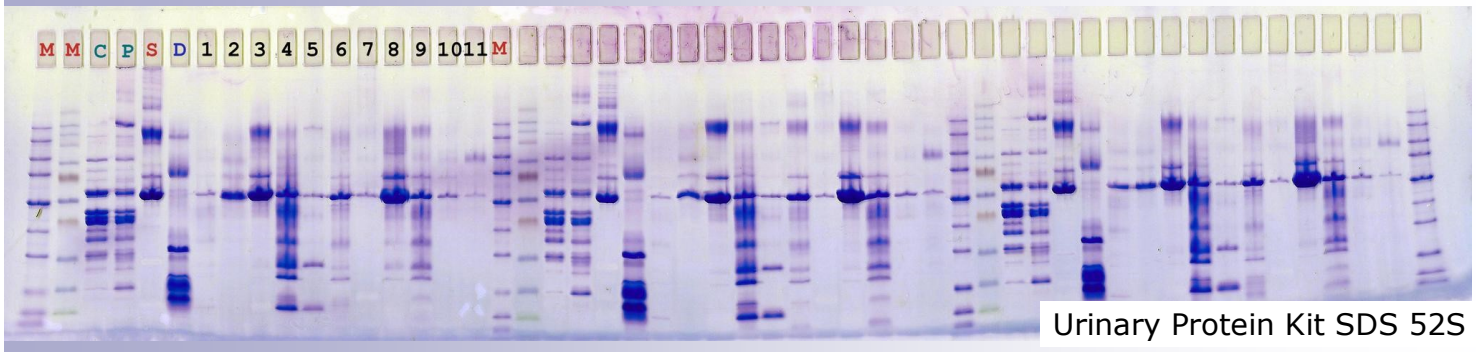
6. Diagnosis of the Results

Running in screaning method: All urins are treated 450 µl + 50 µl Sample Buffer 10X.
Except proteins higher than 2000 mg/l total protein:
These samples are diluted 225 µl Urine + 225 µl H_2O dist. + 50 µl Sample Buffer 10X.



Urinary Protein Kit SDS 24S

Sample	Diagnosis	Protein-Conc.	dilution
mol-weight **standard** *Lonza #50547*	225 kD—5 kD	275 ng per band in 13 µl	100 µl+ 100 µl
1	pure tubulary proteins	143 mg/l	no
2	glomerulary proteins - mid range selectivity	428 mg/l	no
3	glomerulary proteins - no selectivity	2140 mg/l	1+1
4	mixed urinary proteins mostly tubulary	1200 mg/l	no
5	Bence-Jones proteins - free chains	180 mg/l	no
6	mixed urinary proteins	467 mg/l	no
7	native urin	115 mg/l	no
8	mixed urinary proteins mostly glomerulary	2500 mg/l	1+1
9	mixed urinary proteins mostly tubulary + light chains	1082 mg/l	no
10	glomerulary proteins - with selectivity	132 mg/l	no
11	native urin - plus xxx	100 mg/l	no



Urinary Protein Kit SDS 52S