

Cutting-Out PhastGels

General: Cutting eight 'PhastGels IEF' out of a „edc-1110 DryGel IEF“



Cut-out: 50 x 42 mm

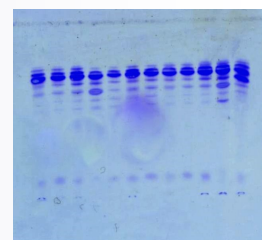


To identify the gel side, breathe on the two sides: the moisture condenses only on the uncoated side!

The cut-out 'PhastGels' do not have a handle!
Please use tweezers to lift the gels (on the longer side) off the cooling plate!

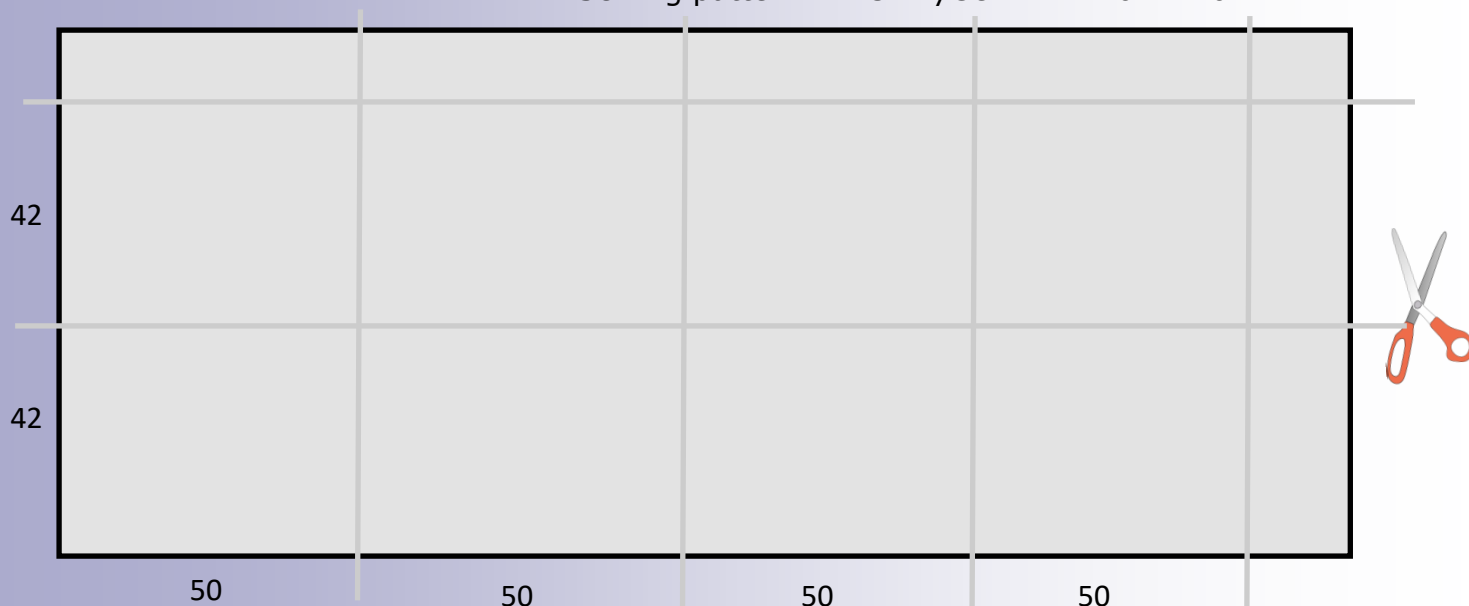


PhastGel

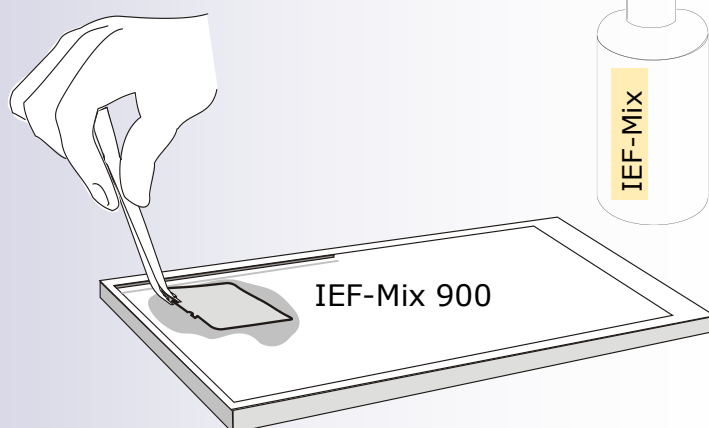
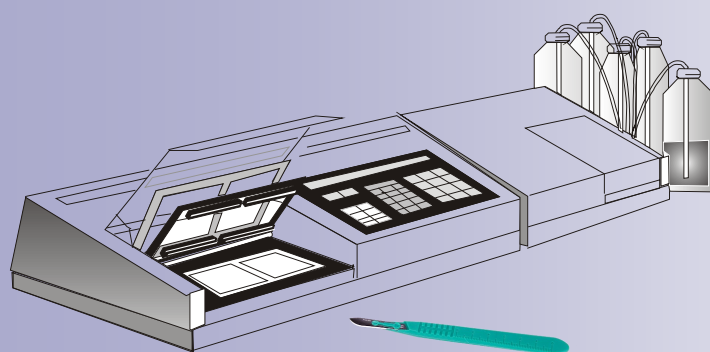
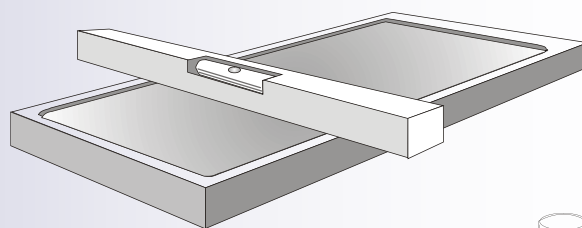
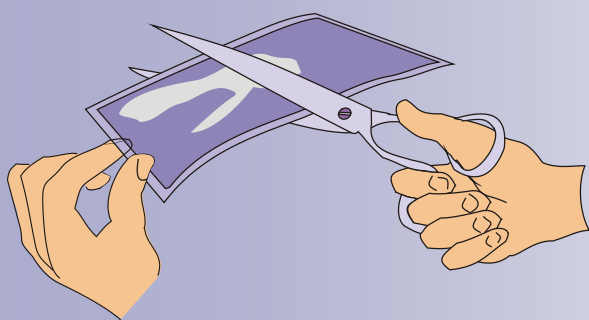


Cut-out

Sewing pattern: EDC DryGel IEF: 240 x 120 mm



Cutting: 8 dry PhastGel IEF out of 1 DryGel IEF



Rehydration for 60-90 min with 800 µl

Rehydration of the Cut-outs

Place the CleanPool or the DryPool Combi (edc-me-d) on a horizontal laboratory table and pipette 900 µl IEF mix into the small compartment or onto the large area of the pool. Make sure that the pipetted volume stays together.

With the gel side down, the cutout is placed on this volume using tweezers.

Rehydration time is 2.5 hours.

To prevent the gel from sticking to the pool, lift the gel several times during this time. Especially in the first 5-10 minutes! Then every 20 minutes.

General recipe for native IEF (example for a gradient pH 3 to 10, 1 ml)

1.5 ml Ampholytes (Servalyte / Biolyte / Sepalyte) pH 3 - 10 (=3% w/v). Addition of 3.5 % (v/v) Ethylenglycol and 1 M urea increases the salt-tolerance of the gel. Fill up to 1 ml with H₂O bidest.

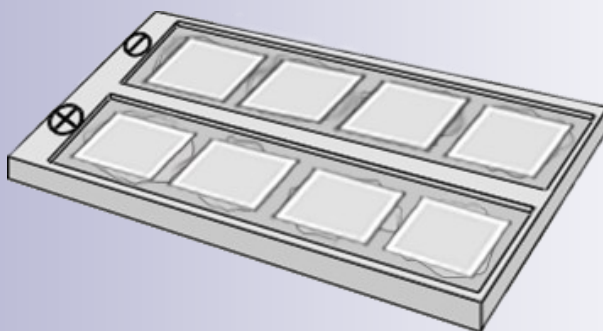
If a special gradient is needed, e.g. pH 4 to pH 5, please note that 10 % of the total Ampholytes should be pH 3 - 10. This prevents that the acid and the alkaline proteins migrate up to the platinum wires.

General IEF Mix for 1 PhastGel (1 ml*)	concentration	volume
Ampholyte (40%)	3% (w/v)*	75 µl
Ethylenglycol	3,5% (v/v)	35 µl
Urea*	1 M	60 mg
H ₂ O	./.	fill up to 1ml

* up to 6% (w/v)
(if needed!)

* not stable!
(only if needed!)

* 900 µl is needed per PhastGel



...or 2 x 4 ‚Phastgels‘ on the backside

Running conditions

Attention: PhastGels from Pharmacia (Uppsala) contained Pharmalytes (discontinued). All other ampholytes (Servalyte, Biolyte etc ...) cannot withstand these running conditions! Therefore, if possible, use these suggested running conditions as a basis.

Runnings	Step	Volt	mA	W	°C	Vh
pre-focusing	1	200	0,2	3,5	15	380
sample entrance	2	200	0,2	3,5	15	15
focusing #1	3	500	0,2	3,5	15	50
focusing #2	4	2000	0,5	3,5	15	200
hold	6	50	0,5	1	10	50

sample applicator COMBS

extra alarm	step 1	300 Vh
appl. down at	step 2	2 Vh
appl. up at	step 2	25 Vh

Special recipes for IEF on Phastsystem

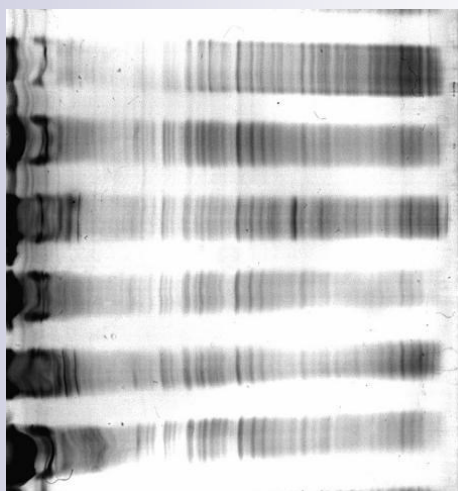
1. Multiple Sclerosis Diagnosis (pH 6-11)

Mix for oligo-IgG (900 µl)	concentration	volume
BioLyte 3/10 (40% w/v)	2.1% (w/v)	47 µl
SinuLyte 3-10 (40% w/v)	0,6% (w/v)	13 µl
Sinulyte 7-9 (40% w/v)	0,13% (w/v)	3 µl
ServaLyte 9-11 (40% w/v)	0,44% (w/v)	10 µl
Ethylenglycol	3,5% (v/v)	30 µl
Urea*	1 M	1,2 g
H ₂ O	./.	to 900 µl

* not stable!

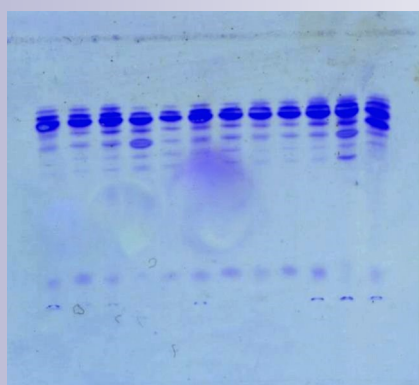
[Manual IEFGel 6-11](#) (big gels)

Running condition: without prefocusing



2. Transferrin Isoforms (pH 5-8)

Mix for Transferrin Isoforms (900 µl)	concentration	volume
BioLyte 3/10 (40% w/v)	0.3% (w/v)	7 µl
SinuLyte 3-10 (40% w/v)	0,1% (w/v)	3 µl
Sinulyte 4-6 (40% w/v)	1,1% (w/v)	25 µl
ServaLyte 4-6 (40% w/v)	1,1% (w/v)	25 µl
BioLyte 5-7 (40% w/v)	0,9% (w/v)	20 µl
Ethylenglycol	3,5% (v/v)	30 µl
H ₂ O	./.	to 900 µl

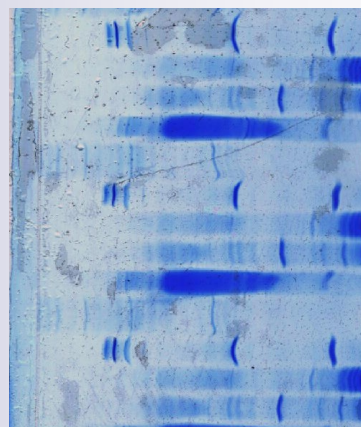


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2. Membran proteins (pH 3-7)

Mix for membrane proteins (900 µl)	concentration	volume
BioLyte 3/10 (40% w/v)	2.1% (w/v)	20 µl
SinuLyte 3-10 (40% w/v)	0,6% (w/v)	10 µl
Sinulyte 3-7 (40% w/v)	1,6% (w/v)	50 µl
ServaLyte 3-7 (40% w/v)	1,6% (w/v)	50 µl
Ethylenglycol	3,5% (v/v)	30 µl
Urea*	7 M	380 mg
H ₂ O	./.	to 900 µl

* not stable!



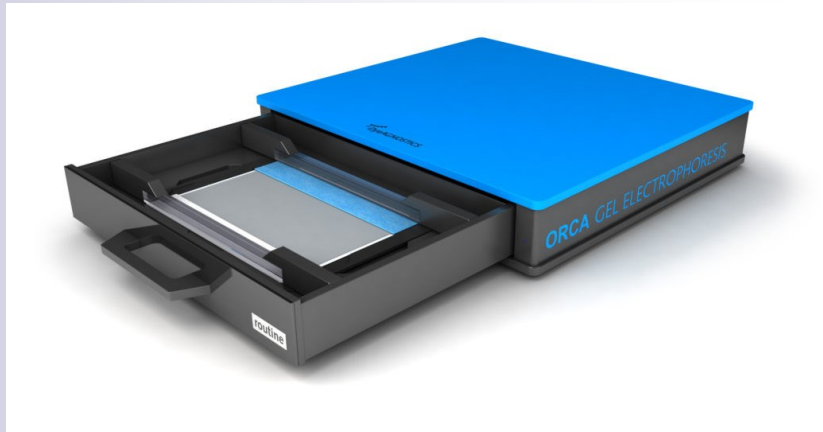
New hardware for automated electrophoresis (incl. fluorescence-dyes and -scanner)

We cooperate with Dyeagnostics GmbH, Halle (Germany).

<https://www.dyeagnostics.com/site/>

See here the information about their complete system: Orca workstation:

<https://www.dyeagnostics.com/site/products/orca-digital-gel-electrophoresis-workstation/>



Electrophoresis unit:

<https://www.dyeagnostics.com/site/en/products/electrophoresis-units/>



Fluorescent Scanner:

<https://www.dyeagnostics.com/site/en/products/electrophoresis-units/>