

ETC Gels – Frequently asked Questions

Focusgel

What are the differences between <i>ETC FocusGel</i> and the GE Ampholine PAGplate?	<i>FocusGel</i> is thinner than PAGplates (0.65 mm): <ul style="list-style-type: none"> - for improved Joule heat dissipation - for faster and more sensitive staining specially treated for catalysts removal used without electrode strips available with and without sample wells
How do the focusing pattern compare?	The pH gradient profiles in an <i>ETC FocusGel</i> are more linear than in an Ampholine PAGPlate.
Which cool contact fluid do I need between cooling plate and film support?	It is highly recommended to use kerosene, because it has no electric conductivity.
Which electrode solutions are used?	None. <i>FocusGel</i> is used without electrode strips
What is the optimal running temperature?	7 °C
Can I apply fluorescent detection methods on a <i>FocusGel</i> ?	There is a special <i>FocusGel NF</i> available, which is polymerised on a low-fluorescent film support.
Is there a <i>FocusGel</i> available for denaturing separation conditions, e.g. containing 8 mol/L urea?	For denaturing conditions you need to rehydrate a <i>CleanGel IEF Ultra</i> in the appropriate urea / carrier ampholyte solution.
Can I perform blotting with <i>FocusGel</i> ?	Yes. It is recommended to use pressure blotting for the protein transfer to the blotting membrane.

Proteomics

IPG-Ampholyte Mix

What is the function of the IPG-Ampholyte Mix?	It is used in the sample extraction and IPG strip rehydration solutions instead of IPG buffers or Pharmalytes™
What is the composition of the IPG-Ampholyte Mix?	It is a mixture of carrier ampholytes with small molecular weights of different origin.
What is the benefit of the IPG-Ampholyte Mix?	Because of the small molecular weights these compounds are washed out more efficiently of the SDS gel during the fixing process. This prevents a dark background in the basic region in Coomassie Blue, silver and fluorescent staining.
What is the concentration in the IPG-Ampholyte Mix?	40 % (w/v), just like IPG buffer or Pharmalytes™.

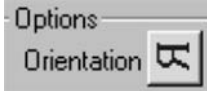
FlapCassette 2D

<i>ETC FlatTop Cassettes 2D</i> and Ettan DALT Precast Gel Cassettes from Amersham / GE Healthcare look very much alike, are they identical?	No. The new <i>ETC FlatTop Cassettes 2D</i> have an improved closing lock, which keeps the cassette better closed.

Vertical Systems (Ettan DALT, Hoefer SE 900, BioRad Dodeca)

General: 2DGel DALT and 2DGel Laemmli

Are these gels compatible with the GE Healthcare DIGE system?	Yes. All 2DGel DALT and 2DGel Laemmli gels with the suffix “ NF ” are polymerised on a non-fluorescent film support.
Are these gels compatible with fluorescent post staining methods?	Yes. All 2DGel DALT and 2DGel Laemmli gels with the suffix “ NF ” are polymerised on a non-fluorescent film support.
What types of 2DGel DALT and Laemmli are available?	Homogeneous 12.5% T and gradient gels 10-15% T. with the suffix NF for fluorescent detection and standard film (with no suffix) support for visible staining methods.
Can I use the Ettan DALT Precast Gel Cassettes from Amersham / GE Healthcare to run these gels?	Yes. There is no problem. However, the new <i>ETC FlatTop Cassettes 2D</i> have an improved closing lock.
Can I run the gels in normal gel casting cassettes of the same size?	No, because there will be mechanical pressure building up on the gels surface leading to a wavy front. Therefore use either <i>ETC FlatTop Cassettes 2D</i> or Ettan DALT Precast Gel Cassettes from Amersham / GE Healthcare, which employ a plastic frame instead of a glass plate on the film-support side.
I have a Hoefer DALT system, will these gels fit into this equipment?	No, they are too large. But we are currently planning to provide gels and cassettes for this system too.
Is the running buffer also available as liquid concentrate?	No. For shipping reasons and to reduce shipping costs the electrode buffers are provided as pre-weighed powder, which dissolves easily and fast in deionised or distilled water.
Do the gels contain a stacking gel?	Yes. They contain a stacking gel zone with 7% T for improved resolution.
Is there a rule for the orientation of the IPG strip?	Yes: It is recommended to apply the IPG strip with the gel surface towards the film support and with the acidic side to the left on the film support: <div data-bbox="1129 1794 1401 1944" data-label="Image"> <p>The diagram shows a hand placing an IPG strip into a cassette. An arrow points to the left edge of the strip, labeled 'acidic end'. Another arrow points to the top surface of the strip, labeled 'IPG strip gel surface up'.</p> </div>
Do I need agarose for overlaying the IPG strip?	No. The strips can be placed directly on the edge of the gel without embedding them with agarose. This prevents also the risk of catching air bubbles between

	SDS gel and the IPG strip.
How can I apply size markers?	Best results are obtained when the size marker proteins have the same start conditions like the proteins from the IPG strips: a IPG strip is rehydrated in the marker protein solution, cut into small pieces, which can be stored in a deep freezer or applied directly to the SDS gel. The alternative is to apply the markers to an IEF sample application piece directly, in a volume of 15 to 20 μ L and let it dry.
What is the optimal running temperature?	20 °C
Can I stain the <i>2DGel DALT NF</i> and <i>Laemmli NF</i> with Coomassie Blue and silver?	Yes and no: It is possible to stain these gels also with hot and/or colloidal Coomassie Blue, but ethanol concentrations above 30 % (v/v) must be avoided. The gels are not suitable for silver staining because of a special treatment of the NF film support. Generally: for visible detection methods like silver and Coomassie Blue staining use the gel types without the “ NF ” specification.
Can I stain a gel with multiple staining procedures?	Yes, The gels are covalently bound to the film support, which protects the gels from swelling and breaking.
How are the gels placed on a scanner?	Directly on the platen with the gel surface down, without contact fluid. In the scanner software choose 
If I cannot scan the gels directly after the run or staining, how should I proceed?	<i>DIGE labelling</i> : Take the gels out of the cassettes, place the provided cover film back on the surface, and store the gels in the provided ZipLog bags in the refrigerator. <i>LavaPurple staining</i> : Keep the gels in the fixing solution or – after staining – in the acidifier over night.
How can I perform blotting with these film-supported gels?	The gels can be easily removed from the support film using the FilmRemover from Amersham / GE Healthcare, or a fishing line or thin wire after mechanically fixing the support film on a round basis, for instance a large bottle.
Are these gels suitable for spot picking and subsequent mass spectrometry analysis?	Yes. These gels have been developed for classical Proteomics workflow, employing an automated spot picker robot. The mass spectrometry results are not influenced by the binding chemistry of the support film.
How can I store the gels between spot detection and spot picking?	Fix the proteins with 10 % acetic acid. Place the provided cover film back on the surface, and store the gels in the provided ZipLog bags in the refrigerator.

2D Gel DALT

Can I run these gels with my	No. These gels contain a special buffer, which has a pH
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standard Tris-glycine running (electrode) buffer?	value below 7 for long shelf life, and enables a very resolving power. Therefore: always use the <i>ETC Buffer Kit 2D DALT</i>
Can I use my standard equilibration buffer for <i>2D Gel DALT</i> ?	No. Because these gels contain a special buffer, it is very important to use the provided equilibration buffer together with urea, DTT and iodoacetamide according to the instructions.
Can I run these gels also in a Single Tank equipment like the BioRad Dodeca or the Hoefer SE 900?	Not recommended. In Single Tank equipment like the BioRad Dodeca or the Hoefer SE 900 the <i>2D Gel Laemmli</i> works better.

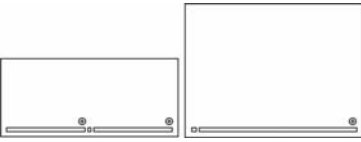
2DGel Laemmli


Can I run these gels with my standard Tris-glycine running (electrode) buffer?	No. These gels contain a special buffer, which has a pH value below 7 for long shelf life, and enables a very resolving power. Therefore: always use the <i>ETC Buffer Kit 2D Laemmli</i> .
Can I use my standard equilibration buffer for <i>2DGel Laemmli</i> ?	Yes. But it is easier to use the provided equilibration buffer together with urea, DTT and iodoacetamide according to the instructions.
Can I run these gels also in a Single Tank equipment like the BioRad Dodeca or the Hoefer SE 900?	Yes. There are gel / buffer kits available specially for this type of equipment.
Can I use the buffer more than once?	It is highly recommended to use new buffer for every run.

Horizontal Systems (Multiphor II and FlatTop)

2D Gel flatbed

Are these gels compatible with the GE Healthcare DIGE system?	Yes. All <i>2DGel flatbed</i> gels with the suffix “ <i>NF</i> ” are polymerised on a non-fluorescent film support.
Are these gels compatible with fluorescent post staining methods?	Yes. All <i>2DGel flatbed</i> gels with the suffix “ <i>NF</i> ” are polymerised on a non-fluorescent film support.
What types of <i>2DGel flatbed</i> are available?	Two different sizes: <ul style="list-style-type: none"> - for two 11 × 11 cm separations and one size marker (fits on Multiphor II and <i>FlatTop</i>) - for one 24 × 18 cm separation and one size marker (fits only on <i>FlatTop Large</i>) homogeneous 12.5%T and gradient gels 10-15%T. with the suffix <i>NF</i> for fluorescent detection and standard film (with no suffix) support for visible staining methods.
Do the gels contain a stacking gel?	Yes. They contain a stacking gel zone with 7%T for improved resolution. The slot for the IPG strip is located inside the stacking zone.
What is the benefit of a horizontal <i>2DGel flatbed</i> ?	Easy handling, easy cleaning of equipment, small buffer volumes, efficient cooling, thin gel layer for

	faster and sensitive staining. High flexibility when running only few separations. For small format: two separations in one gel.
What is the only disadvantage of horizontal 2D gel systems compared to vertical systems?	The IPG strip has to be removed from the gel before the high field strength (high voltage) phase begins, because the immobilised charges in the IPG strip will cause surface water transport resulting in drying out of the gel. This does not happen in a vertical system, because those are closed systems.
Why have horizontal gel systems not been more used so far?	Because the protein transfer from the IPG strip to the second dimension had been less efficient than for vertical gels. But <i>2DGel flatbed</i> does not have this disadvantage.
What is the special feature of <i>2DGel flatbed</i> ?	These gels are prepared by a special procedure, which allows polymerising them with a slot(s) in the stacking zone for accommodating the IPGstrip(s). In this way the protein transfer is as efficient as for vertical gels.
How important is it to remove the IPG strip from the gel after 1 hour 20 minutes?	It is highly recommended to remove the IPG strips before the high field strength (high voltage) phase begins, because the immobilised charges in the IPG strip will cause surface water transport resulting in drying out of the gel. This does not happen in a vertical system, because there are no open surfaces.
Here I can only run one gel at once, how can I run more than one <i>2DGel flatbed</i> parallelly?	There is a <i>FlatTop Tower</i> available for running up to four gels parallelly.
Does the provided running buffer need to be diluted?	No. It is used in its concentrated form by soaking the electrode paper wicks in it.
Which cool contact fluid do I need between cooling plate and film support?	Just the cathode buffer. It spreads out very well, because it contains SDS. It is not recommended to use kerosene or any other cool contact fluid.
What is the optimal running temperature?	15 °C
Can I use my standard equilibration buffer for <i>2DGel flatbed</i> ?	No. Because these gels contain a special buffer, it is very important to use the provided equilibration buffer together with urea, DTT and iodoacetamide according to the instructions.
Is there a rule for the orientation of the IPG strip?	Yes: It is recommended to apply the IPG strip with the gel surface down in the slot and with the acidic side to the right, when the cathode and the slot is on the side of the operator: 
How can I apply size markers?	The gel contains a small well in the gel surface for 5 µL marker protein solution.
Can I stain the <i>2DGel DALT</i> and <i>Laemmli NF</i> with Coomassie Blue and silver?	Yes and no: It is possible to stain these gels also with hot and/or colloidal Coomassie Blue, but ethanol concentrations above 30 % (v/v) must be avoided. The gels are not suitable for silver staining because of a

	<p>special treatment of the NF film support. Generally: for visible detection methods like silver and Coomassie Blue staining use the gel types without the “NF” specification.</p>
Can I stain a gel with multiple staining procedures?	Yes, The gels are covalently bound to the film support, which protects the gels from swelling and breaking.
How are the gels placed on a scanner?	<p>Directly on the platen with the gel surface down, without contact fluid. In the scanner software choose</p> 
If I cannot scan the gels directly after the run or staining, how should I proceed?	<p><i>DIGE labelling:</i> Take the gels out of the cassettes, place the provided cover film back on the surface, and store the gels in the provided ZipLog bags in the refrigerator.</p> <p><i>LavaPurple staining:</i> Keep the gels in the fixing solution or – after staining – in the acidifier over night.</p>
How can I perform blotting with these film-supported gels?	The gels can be easily removed from the support film using the FilmRemover from Amersham / GE Healthcare, or a fishing line or thin wire after mechanically fixing the support film on a round basis, for instance a large bottle.
Are these gels suitable for spot picking and subsequent mass spectrometry analysis?	Yes. These gels have been developed for classical Proteomics workflow, employing an automated spot picker robot. The mass spectrometry results are not influenced by the binding chemistry of the support film.
How can I store the gels between spot detection and spot picking?	Fix the proteins with 10 % acetic acid. Place the provided cover film back on the surface, and store the gels in the provided ZipLog bags in the refrigerator.