

ETC Gels – Frequently asked Questions

Proteomics Horizontal

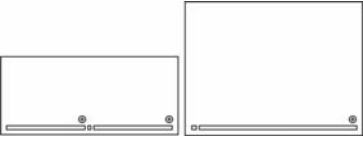
IPG-Ampholyte Mix


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™.

Horizontal Systems (Multiphor II and FlatTop)

2DGel flatbed

Are these gels compatible with the GE Healthcare DIGE system?	Yes. All 2DGel flatbed 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 flatbed gels with the suffix “ NF ” are polymerised on a non-fluorescent film support.
What types of 2DGel flatbed 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 NF 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 2DGel flatbed ?	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 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

	Options Orientation 
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.