

## ETC Gels – Frequently asked Questions

### *Proteomics Vertical*

#### 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™.

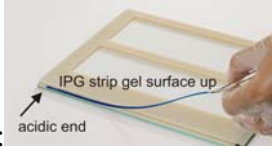

#### FlapCassette 2D

<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 <i>2DGel DALT and 2DGel Laemmli</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 DALT and 2DGel Laemmli</i> gels with the suffix “ <i>NF</i> ” are polymerised on a non-fluorescent film support.
What types of <i>2DGel DALT and Laemmli</i> are available?	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.
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: 
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 $\mu$ L and let it dry.
What is the optimal running temperature?	20 °C
Can I stain the 2DGel DALT NF and Laemmli NF 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.

## 2DGel DALT

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 2DGel 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.