



PRODUCTION OF CONSISTENTLY HIGH-QUALITY AFFORDABLE EMULSIONS WITH FREUND'S ADJUVANT & PRECELLYS HOMOGENIZERS

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CONTEXT

The development of new vaccines and drugs often requires an emulsion step prior to injection. This is also the case of autoimmune diseases and immunotherapy treatments for cancer. In these approaches, the emulsion step is key to the experiment success. For example, to induce disease in the human models for Multiple Sclerosis (EAE) and Rheumatoid Arthritis (CIA), emulsions must be water-in-oil, stable and particle size should be homogenous. Unfortunately, current methods to prepare emulsions have several limitations, among which:

- Reproducibility: high quality emulsions are difficult to generate consistently due to person-to-person variations;
- Throughput: traditional methods are time consuming and only allow to produce one emulsion at the time
- Cost efficiency: due to important dead volume, a large quantity of drugs can be lost.

In order to overcome these difficulties, BTB Emulsions has developed a device and method to be used on Precellys homogenizers. This new Precellys Emulsion kit ensures the generation of several high-quality emulsions in a consistent way.

MATERIALS

- Minilys homogenizer, up to 3 emulsions can be prepared in less than 10 minutes
- Precellys Emulsion kit: 10 sterile, individually packed devices (D34200.10 ea)





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PROTOCOL

- To prepare high-quality, reproducible emulsions with Bertin homogenizers and Precellys Emulsion kit:
 - Place receptacle on ice and add PBS, antigen, and other aqueous solutions.
 - Add the adjuvant (Freund's Montanide, etc..)
 - Shake receptacle for 5s by hand
 - Shake with Bertin homogenizers >> with Minilys: 1min cycle at 5000 rpm, place tube on ice 3min then repeat 1min cycle at 5000 rpm
 - Centrifuge the receptacle for 1 minute at 300g to recover all the emulsion
- Visit https://youtu.be/uFl6hY-j1_I for a full protocol (video)

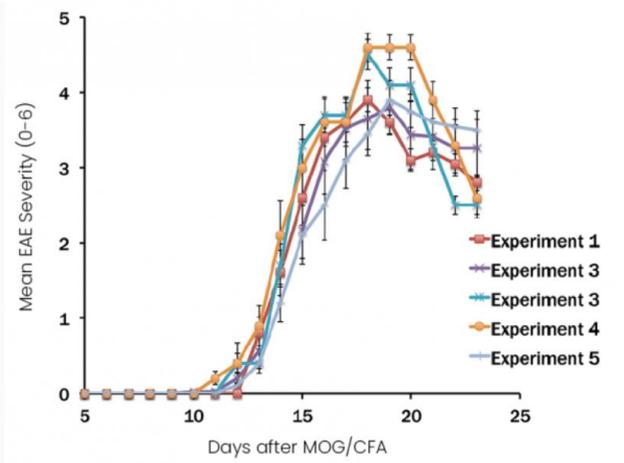
RESULTS

Figure 1: BrightField (400x) images of emulsions prepared using the Minilys and Precellys Emulsion kit method, traditional 2-syringe method, or Vortex. Emulsions prepared with Minilys and Precellys Emulsion kit are stable and have homogenous particle size. Emulsions prepared with Vortex, do NOT induce EAE (results not shown).



Figure 2: Induction of disease in the animal model for human Multiple Sclerosis, EAE. Emulsions prepared with Minilys and Precellys Emulsion kit consistently induce EAE.

Emulsions were prepared fresh each time, using Precellys Emulsion kit. Five different experiments were set up, at least 1 month apart. Mice, C57BL/6J, were bought from Janvier and were delivered 1 week before each experiment (5 in each group).





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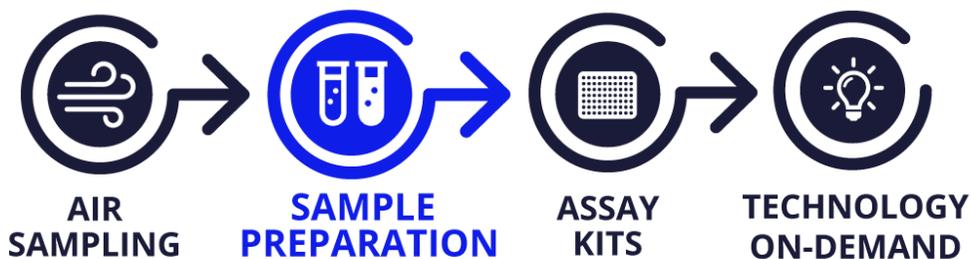
CONCLUSION

BTB Emulsions and Bertin Technologies introduce a new solution using Bertin Technologies homogenizers and Emulsion kits to standardize the preparation of emulsions with Freund's Adjuvant. With Minilyls and Emulsion kits, one can prepare up to 3 perfect emulsions in less than 10 minutes.

The resulting emulsions are stable and homogenous in terms of particle size, and consistently induce EAE and CIA in these human models. This technology is time saving, user friendly, quality controlled, and unlike any other method, it produces a high-quality emulsion every time. It is also compatible with other oil-based adjuvants such as Montanide™.



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