



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

BTB Emulsions AB, Malmö, Sweden

/ INTRODUCTION

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 named POWER-Kit™. This combination 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 Evolution homogenizer, up to 12 emulsions can be prepared in less than 10 minutes
- POWER-Kit™: 10 sterile, individual packed devices from BTB Emulsions

/ PROTOCOL

To prepare high-quality, reproducible emulsions with Bertin homogenizers and BTB Power-Kit™:

- 1) Place receptacle on ice and add PBS, antigen, and other aqueous solutions.
- 2) Add the adjuvant (Freund's Montanide, etc..)
- 3) Shake receptacle for 5s by hand
- 4) Shake with Bertin homogenizers:
 - With Minilys: 1min cycle at 5000 rpm, place tube on ice 3min then repeat 1min cycle at 5000 rpm
 - With Precellys Evolution: 1min cycle at 7300 rpm, place tube on ice 3min then repeat 1min cycle at 7300 rpm
- 5) Centrifuge the receptacle for 1 minute at 300g to recover all the emulsion

Visit www.btbemulsions.com for a full protocol (written & video)

/ RESULTS

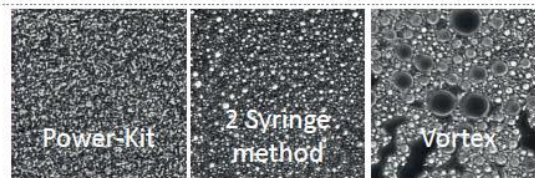


Figure 1: Brightfield (400x) images of emulsions prepared using the Minilys and POWER-Kit™ method, traditional 2-syringe method, or Vortex. Emulsions prepared with Minilys and POWER-Kit™ are stable and have homogenous particle size. Emulsions prepared with Vortex, do NOT induce EAE (results not shown).

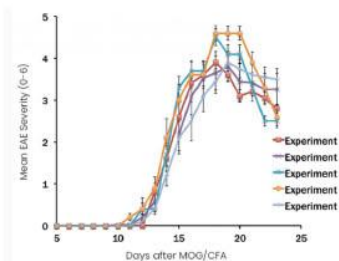


Figure 2: Induction of disease in the animal model for human Multiple Sclerosis, EAE

Emulsions prepared with Minilys and POWER-Kits™ consistently induce EAE.

Emulsions were prepared fresh each time, using POWER-Kits™. 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).

/ CUSTOMER



CONTACT
Email: btb@btbemulsions.com
www.btbemulsions.com

/ CONCLUSION

BTB Emulsions has introduced a new solution using Bertin Technologies homogenizers and the POWER-Kit™ to standardise the preparation of emulsions with Freund's Adjuvant. With Minilys and POWER-Kit™, one can prepare up to 3 perfect emulsions in less than 10 minutes. With Precellys Evolution, up to 12 emulsions can be produced 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™.



□東京 〒162-0805 東京都新宿区矢来町 113 番地 TEL (03)3235-0661(代) / FAX (03)3235-0669
□大阪 〒532-0005 大阪市淀川区三国本町2丁目12番4号 TEL (06)6396-0501(代) / FAX (06)6396-0508

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