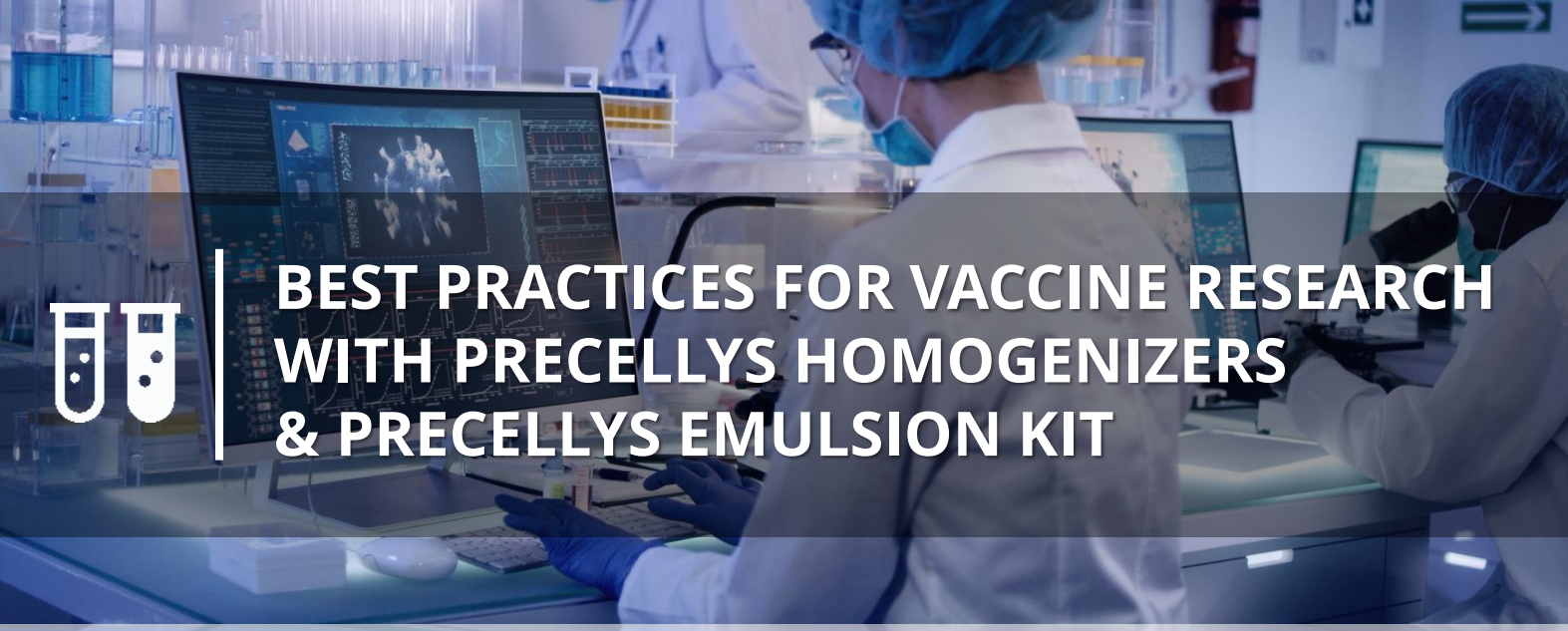


BEST PRACTICES FOR VACCINE RESEARCH WITH PRECELLYS HOMOGENIZERS & PRECELLYS EMULSION KIT

Vaccination is largely recognized as the most cost-effective way to fight human infectious diseases. Vaccines play a vital role in immunization strategies that have contributed to eradicate dangerous pathogens, increase human life expectancy and reduce disabilities & suffering all around the world. Because both prophylactic and therapeutic vaccination can help reach herd immunity, vaccine research will be crucial in maintaining public health in the future. The majority of vaccine studies still rely on the use of animal models, in which animals are immunized with the candidate vaccine and then exposed to the pathogen of interest. Most often, the efficiency of the vaccine candidate is assessed with the help of molecular biology techniques that require the homogenization of the infected animal tissues. Bertin has developed a range of homogenizers, the Precellys, to offer researchers a reproducible, robust solution to safely homogenize infected animal tissues and obtain high-quality molecules, compatible with modern molecular biology analysis workflows. The Precellys are powered by 3-D bead-beating, a state-of-the-art technology that is widely acknowledged as the golden standard for homogenization.

A great number of vaccines are also based on the use of adjuvants, substances whose role is to boost the immune system's reaction to an antigen. Currently, many adjuvants are emulsion-based, such as the Freund's complete adjuvant or the MF59. Unfortunately, most of the existing methods to prepare emulsions suffer from several pitfalls, including low reproducibility, low throughput, and low cost-efficiency. Bertin Technologies presents the Precellys Emulsion kit, a new turnkey solution to generate high-quality emulsions with the Precellys homogenizers in a consistent way.

In this white paper, we show several application notes where the Precellys homogenizers are used to study infected tissues, to obtain high-quality nucleic acids but also a high recovery of viable microorganisms. We also show how the Precellys Emulsion kit can be used with the Precellys homogenizers to deliver reproducible, high-quality emulsions.

BOOST YOUR VACCINE STUDIES WITH THE PRECELLYS HOMOGENIZERS & THE PRECELLYS EMULSION KIT

SUMMARY

RECOVER LIVE HERPES VIRUS FROM INFECTED MOUSE GANGLIA WITH MINILYS

kwang@niaid.nih.gov, MVS, LID, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

/ CONTEXT

The research focus of our laboratory is on human herpes virus pathogenesis and the development of new treatments and vaccines. We often need to test herpes simplex viruses (HSV) and their mutants in a mouse model to study the function of viral genes, evaluate antiviral therapies, and assess the efficacy of vaccine candidates. The titer of infectious HSV in the sensory nerve ganglion innervating the inoculation site is a crucial measurement for these studies.

/ MATERIALS

- Minilys homogenizer.
- Precellys lysing kit: 03961-1-203 (CK14 - 0.5mL tubes).
- HSV-2 virus stock with known titer.
- Mouse trigeminal ganglia (TG) harvested three days after HSV-2 cornea infection.
- Medium HBSS with 1 % FBS.

/ PROTOCOL

- Virus stock was diluted to desired concentration and 500 µl was transferred to a homogenizer tube.
- A fresh TG was placed in a homogenizer tube with 500 µl of medium.
- All above samples were kept on ice unless specified.
- Samples were homogenized at RT with settings as indicated (see Figures 1 and 2). Homogenization was paused every 20 seconds and tubes were held on ice for 1 minute then continue homogenization.
- Live virus in homogenates was detected by titration on a Vero cell monolayer in 6-well-plates following standard plaque assay protocol.

/ RESULTS

The mouse TG tissue required more than 3 k rpm x 45 sec or 4 k rpm x 20 sec to be adequately homogenized (results not shown).

Diluted virus stock (cell free virus) can withstand the homogenization up to 5 k rpm x 60 sec (Fig. 1). Live virus can be recovered from HSV-2 infected mouse TG (Fig. 2).

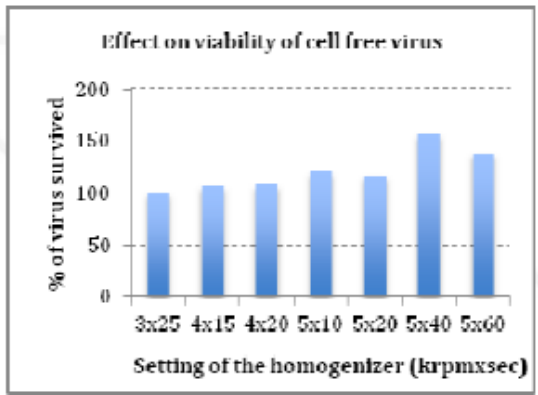


Figure 1

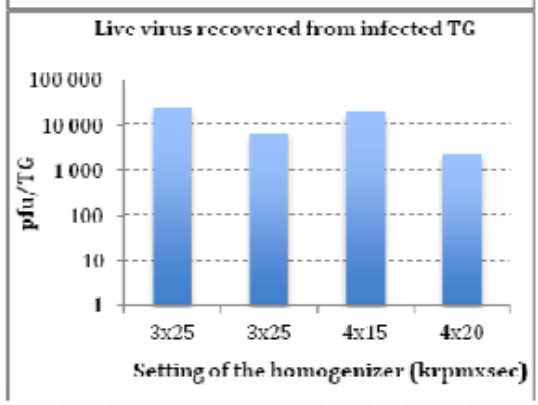


Figure 2

Live herpes simplex virus can be effectively recovered from infected mouse tissue with Minilys by homogenizing infected tissue in individual, tightly-closed tubes rather than in open tubes with a rotor-stator homogenizer.

This facilitates medium throughput analysis of tissue samples and avoids the possibility of cross-contamination between samples.

03712-810-DU065

MAINTAINING BACTERIAL VIABILITY DURING TISSUE HOMOGENIZATION

Illawarra Health and Medical Research Institute, University of Wollongong, Australia

/ CONTEXT

In an animal model of invasive infections caused by Streptococcus pyogenes, bacteria will colonize various tissues and organs. Bacterial loads present in these tissues is determined by homogenization, serial dilution and plating onto nutrient agar.

This study investigates the use of the Precellys 24 for tissue homogenization.

/ MATERIALS

- - Precellys 24 homogenizer.
- - Precellys kits: 03961-1-003 (1.4mm ceramic beads) and 03961-1-002 (2.8mm ceramic beads).
- - Samples: skin (122.3 mg) and spleen tissues (38.6 mg).
- - Bacteria culture: S. pyogenes was grown in Todd Hewitt
- Broth supplemented with 1% yeast extract at 37°C un til an
- A 600= 0.5.
- - Buffer: 0.7% sterile saline (1000µl) inoculated with S.pyogenes (T0 Inoculum).

/ PROTOCOL

Note that pathogenic S. pyogenes must be processed under appropriate biosafety containment.

- Precellys24 parameters:
- Spleen: 6000 rpm, 1x30 sec,
- Skin: 6000 rpm, 2x30 sec or 6000 rpm, 4x30 sec.
- Analysis: assessment of S.pyogenes concentration (CFU/ml) by cultural method.

/ RESULTS

Following tissue homogenization, the number of viable bacterial cells (CFU/ml) present was determined by serial dilution in sterile 0.7% saline and plating onto nutrient agar.

Results (see Figure1) indicate that for all treatment groups the type of bead and the processing time had no significant effect on the viability of S. pyogenes (t test p> 0.1).

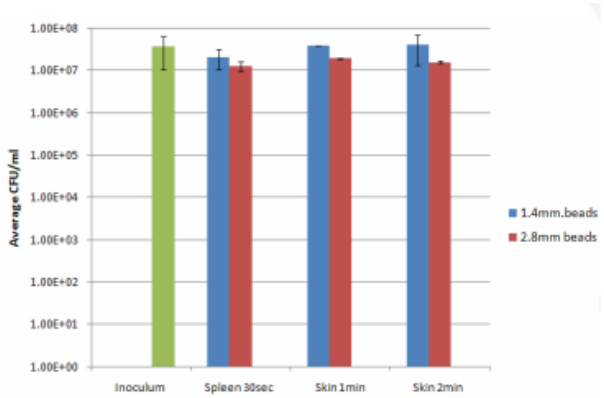


Figure 1: The effect of tissue homogenization on the viability of S. pyogenes. Data represents the mean CFU/ml from triplicate samples with error bars indicating the standard derivation. The inoculum represents the CFU/ml added to each sample.

Tissue homogenization (spleen & skin) using the Precellys 24 with ceramics beads kits (1.4mm or 2.8mm) does not affect the viability of the Gram positive pathogen S.pyogenes present in these samples. The bacterial viability is maintained during tissue homogenization.

The potential to process up to 24 individual samples in a short time period with no risk of cross-contamination has considerable benefits over traditional homogenization methods.

03712-810-DU053

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

BTB Emulsions AB, Malmö, Sweden

/ 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)

/ PROTOCOL

To prepare high-quality, reproducible emulsions with Bertin homogenizers and Precellys Emulsion 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

5) Centrifuge the receptacle for 1 minute at 300*g* 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).

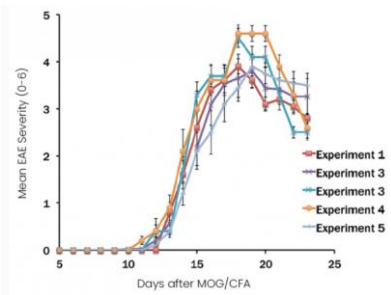


Figure 2: Induction of disease in the animal model for human Multiple Sclerosis, EAE. Emulsions prepared with Minilys and Precellys Emulsion kit consistently induce EAE.

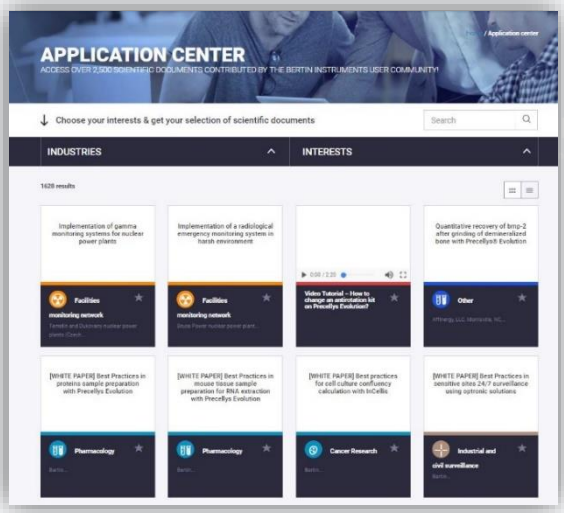
Emulsions were prepared fresh each time, using Precellys Emulsion kit. Five different experiments were set up, at least 1 month apart. Mice, C57BL/6J, were bought from Janvier and were delivered 1 week before each experiment (5 in each group).

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 Minilys and Emulsion kits, one can prepare up to 3 perfect emulsions in less than10 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™.

PREC-0266DU146-D

Use the Application Center to find the appropriate protocol for your application & optimize it with users feedback!

- Find scientific documents
- Find the appropriate kit
- Share with the Precellys® community



Precellys® Evolution: the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs

- **Flexibility:** 24 x 2mL (or 0,5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with the Cryolys® Evolution cooling unit

Bertin Bioreagent: a comprehensive offer of best in class analysis tools dedicated to biomarkers

- **Commitment to quality & guaranteed performance**
- **30 years of know-how** in biomarker assays
- **A complementary reagent range** for streamlined workflows with Bertin Instruments

Liste des équipements

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