University of Zurich[UZH] Institute of Laboratory Animal Sciences	**Standard Operating Procedure** **SOP**	**Page 1 of 6**
Date: 23.05.2022	**Active EAE induction**	**LTK-RES-26-EN** **Version: B**

This SOP replaces:	Date: 02.08.2018 Version: A
Reason for Change:	To clarify certain parts of the previous SOP, i.e., material preparation
Related SOPs:	SOP-LTK-RES-1-EN EAE Scoring SOP-LTK-TRT-10-EN ip injection SOP-LTK-TRT-11-EN sc injection SOP-LTK-TRT-13-EN Isoflurane anesthesia SOP-LTK-TRT-25-EN Marking and Biopsies
Indication of Use:	Induction of active Experimental Autoimmune Encephalomyelitis (EAE)
Aim of SOP:	This protocol describes how to induce mice with active EAE by co-administration of peptide, CFA and Pertussis toxin
Distribution:	1. Original: Server 2. Copy: Animal Facility 3. Intranet
Attachments:	

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Responsible Persons: Researcher with Module 1 education and on respective permit

Method

Prepare 5 mg/mL peptide (example MOG_{35-55}) stock solution
Dissolve 30 mg of MOG in 1 mL of deionized water.
Add another 5 mL of deionized water.
The solution should be completely transparent at this point.
Make 500 µL aliquots and keep it at -80 °C until use.

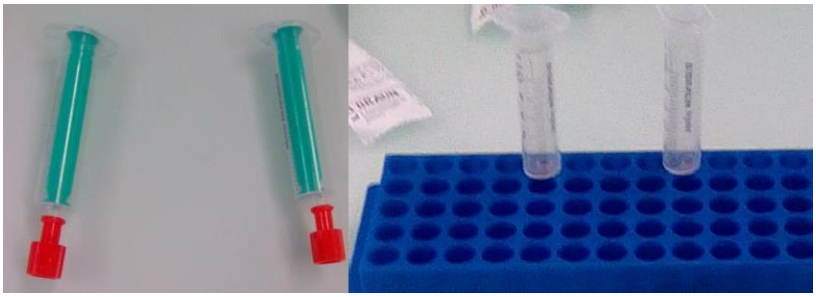
Prepare CFA+ adjuvant solution
Each mouse should receive an added 500 ug of *Mycobacteria tuberculosis* (250 ug per injection site). Therefore, the final adjuvant solution should contain *Mycobacteria tuberculosis* at 2,5 mg/ml in CFA. For this, add 25 mg of desiccated *M. tuberculosis* into 10 mL of CFA. Keep it in the fridge until use. Note that the extra *M. tuberculosis* will not dissolve and a portion of it will remain in suspension and deposit in the bottom of the vial.

Explained: Explained: extra 250 ug per injection site, 100 µL per injection site, 2 sites → 500 ug/200 µL = 2.5 mg/mL, in 10 mL CFA→ 25 mg of *Mycobacteria tuberculosis*

Prepare 2 µg/mL Pertussis Toxin aliquots
As soon as the Pertussis Toxin arrives, make 10 µL aliquots from the stock solution and keep it at -20 °C until use.

Prepare working emulsion
At Day 0
dilute stock peptide (e.g., MOG_{35-55} in deionized water) in PBS, so that the final concentration of MOG_{35-55}-dH20 in the final volume of emulsion is 1 mg/mL.
Add the same amount of CFA+ (vortex the CFA+ solution prior to adding, so that the *M. tuberculosis* particles are homogeneously distributed throughout the solution).

Prepare the syringes as shown below. Note that the plunge must be taken out before attaching the red adaptors. Place the syringes and the properly attached red adaptors on a microtube rack and pipette the MOG in PBS solution into one syringe and the CFA+ into the other.
Re-insert the plunges, paying attention that the solutions do not spill. Release the red cap slowly and get rid of any air bubble. Do not apply too much pressure in the plunge or the solutions may squirt.
When there are no air bubbles in both preparations, attach the metal adaptor.



Mix the solutions by passing them from one syringe to the other at a constant rate for at least 20 min.
The final solution must not have any air bubbles and should be homogeneous and white.

Example
We need to inject 8 mice with the emulsion.

Note: Always prepare the emulsion in excess (e.g., For 8 mice → calculate for 10, as much is lost)

Emulsion

We inject 200 ul/mouse (100 ul per site)

Eg. For 10 mice, the final V of the emulsion is 200ulx10=2000 ul=2 ml

As a rule, half of the volume is CFA+ and the other half is (MOG in PBS).

CFA+: 50 ug inactivated Mycobacteria in 10 ml CFA (C= 2,5 mg/ml in 10 ml CFA)

V_{CFA}= Vfinal/2 → 2ml/2= 1 ml CFA

MOG: Stock: 5mg/ml in deionized water, we use it at 1 mg/ml in the final emulsion volume

$V_{MOG\ in\ PBS}$= Vfinal/2 → 2ml/2= 1 ml MOG in PBS

For V_{MOG} (from stock solution): C1*V1=C2*V2 → 5 mg/ml* V1= 1 mg/ml * 2 ml → V1= 0,4 ml
→ The remaining volume to reach 1 ml will be filled with PBS → V_{PBS}= 0,6 ml

In this way, V_{PBS} can also be confirmed by: $V_{PBS}=V_{final}- V_{CFA}- V_{MOG}$= 2- 1- 0,4= 0,6 ml

In this way, we would use:

400 ul MOG in deionized water (stock solution) diluted in 600 ul PBS (=1 ml total) & 1 ml CFA+, making a total of 2 ml emulsion volume, and emulsify them using the syringes as shown above.

Pertussis Toxin: Stored in 10 ul aliquots

Stock= 0,2 mg/ml

We need it at: 6,66 ng/ul in PBS for day 0 and day 2 (prepare fresh each day)

We inject 100 ul/mouse/day

Eg. For 8 mice (prepare for 8 mice exactly): C1*V1=C2*V2 → V1= 6,66 ng/ul*0,8 ml/ 0,2 mg/ml → V1= 6,66 ng/ul*0,8 ml/ 0,2x10^6 ng/10^3 ul= 26,64 ul in 800 ul PBS

Induce mice
At day 0

1. Anesthetize mice with isofluorane (SOP-LTK-TRT-13-EN Isoflurane anesthesia)
2. Weigh each mouse prior to emulsion administration
3. If needed, ear mark the mice while under anesthesia (SOP-LTK-TRT-13-EN Isoflurane anesthesia)
4. Immunize subcutaneously with emulsion (SOP-LTK-TRT-11-EN sc injection), on either side of the midline on the lower back (Use 100 µL of emulsion per injection site → total of 200 µl)
5. Inject 100 µL of Pertussis Toxin i.p (SOP-LTK-TRT-10-EN ip injection)

At day 2

1. Weigh each mouse prior to injection
2. Inject 100 µL of Pertussis toxin i.p (SOP-LTK-TRT-10-EN ip injection)

Reagents:
MOG35-55 (MEVGWYRSPFSRVVHLYRNGK), GenScript Cat. No. RP10245 t for C57BL/6
PLP139-151 (HSLGKWLGHPDKF), Genescript Cat. No. RP10550 for SJL
MBP Ac1-11 (ASQKRPSQRSK) Genescript Cat. No. RP20350 for PL/J, B10.PL
MOG1-15 (GQFGVIGPGYPIRAL) Genescript custom order for AB/H Biozzi
Ovalbumin, SigmaAldrich, Cat. No. A5503
$OVA_{257-264}$ (SIINFEKL), GenScript Cat. No. RP10611
$OVA_{323-339}$ (ISQAVHAAHAEINEAGR), GenScript Cat. No. RP10610-1
KLH native protein, Abcam Cat. No. ab285712
Hemagluttinin (HA) peptide (YPYDVPDYA), GenScript Cat. No. RP11735
Chicken gamma globulin (CGG), Jackson ImmunoResearch Cat. No. 003-000-002
MOG, PLP, MBP, Aquaporin and other proteins (for antigen lists and concentrations see Literature section)

Complete Freund's Adjuvant (CFA) H37 Ra (BD™ 231131; DF3113-60-5, Thermo Fisher)
Mycobacteria tuberculosis H37 Ra (avirulent, disssecated) (BD™ 264011; DF3114-33-8, Thermo Fisher)
Pertussis Toxin in Glycerol (List BioLabs, #179B)

Material:
1 mL syringes
For Pertussis toxin administration – 1 mL Tuberculin – 9166017 V (Braun)
For emulsion administration – 1 mL Conventional Insulin syringes – 309629 (BD, Luer-Lok)
2 mL or 5 mL Luer lock syringes
e.g. 2 mL Norm-Ject – Cat # 1481727 (Fischer scientific)
5 mL Luer Lock Solo – 4606710V (Braun)
22 G needles 0.70 x 30 mm (Braun)

Safety:
General lab safety rules apply.
Use goggles when preparing and handling the emulsion.

Factors influencing outcome:
Pertussis toxin batch may influence severity of disease.
Homogeneity of emulsion.
Mice need to be acclimatized to the animal facility.

Criteria for approving outcome:
In knockout experiments, use WT mice as positive control.

Analysis:
EAE scoring (as per SOP)

Documentation:
Lab book, server.

Literature:

1. Amor, S., et al., *Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice.* J Immunol, 1994. 153(10): p. 4349-56.
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3. Al-Izki, S., et al., *Practical guide to the induction of relapsing progressive experimental autoimmune encephalomyelitis in the Biozzi ABH mouse.* Mult Scler Relat Disord, 2012. 1(1): p. 29-38.
4. Racke, M.K., *Experimental autoimmune encephalomyelitis (EAE).* Curr Protoc Neurosci, 2001. Chapter 9: p. Unit9 7.
5. Miller, S.D. and W.J. Karpus, *Experimental autoimmune encephalomyelitis in the mouse.* Curr Protoc Immunol, 2007. Chapter 15: p. Unit 15 1.
6. Croxford, A.L., F.C. Kurschus, and A. Waisman, *Mouse models for multiple sclerosis: historical facts and future implications.* Biochimica et biophysica acta, 2011. 1812(2): p. 177-83.
7. Meng, Y., Ding, L., Zhang, H.Y., Yin, W.C., Yan, Y. and Cao, Y.P., 2017. An Aβ3-10-KLH vaccine reduced Alzheimer's disease-like pathology and had a sustained effect in Tg-APPswe/PSEN1dE9 mice. *Brain Research*, *1673*, pp.72-77.