
 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 1 of 8
Date: 20.09.2018	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-F-EN Version: F

This SOP replaces:	Version: E
Reason for Change:	Small changes in wording
Related SOPs:	Induction of EAE (LTK-RES-26-A-EN)
Indication of Use:	Scoring of mice after active Experimental Autoimmune Encephalomyelitis (EAE) has been induced (e.g. by immunization with myelin antigen or by adoptive transfer of immune cells); scoring of mice which develop EAE spontaneously (e.g. 2D2/IgHMOG mice); scoring of mice in which myelin components are ablated by chemical or genetic means
Aim of SOP:	Mice are scored to obtain a defined measures of disease severity as experimental data point. Further this protocol serves as criterion for the application of measure for animal welfare. This protocol determines also the termination points when an animal needs to euthanized.
Distribution:	1. Server
Attachments:	
Generated at: 20.09.2018	Checked and approved at: 20.09.2018
by: Thorsten Buch	by: Dr. Prajwal

Responsible Persons: Researcher with Modul 1 after registration on animal license including EAE protocols

Method: Physical test and observation by the researcher, weight

SOP-LTK-RES-1-F-EN EAE Scoring.docx

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 2 of 8
Date: 20.09.2018	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-F-EN Version: F

Principle of Method: observation, weighing

Units and Formulas:

Clinical scoring, weight and observation


Score	Clinics	Assessment	Action
0	No detectable signs of EAE		
0.5	Distal limp tail		
1.0	Complete limp tail		
1.5	Limp tail and hind limb weakness	Occasional grid test positive	From now on provide chow and gel packs in the cage (separate)
2.0	Unilateral partial hind limb paralysis	One leg consistently falls through the grid (cage test)	
2.5	bilateral partial hind limb paralysis	Cage grid test bilaterally positive	
3.0	Complete bilateral hind limb paralysis		Sacrifice if persistent >7 days
3.5	Complete bilateral hind limb paralysis and partial forelimb paralysis		Sacrifice if persistent >3 days
4.0	Moribund (mouse completely paralyzed)		Sacrifice immediately
5.0	dead		

On each observation day the weight is recorded and documented (in g)

Material to be used: Cage top, grid

Machine: Scale, laminar flow/changing station

Safety:
Follow the rules for the respective animal room

 <p>University of Zurich^{UZH} Institute of Laboratory Animal Sciences</p>	<p align="center">Standard Operating Procedure</p> <p align="center">SOP</p>	<p align="center">Page 3 of 8</p>
<p>Date: 20.09.2018</p>	<p align="center">Scoring of Mice after EAE Induction EAE Scoring</p>	<p>LTK-RES-1-F-EN Version: F</p>

Method Description:

1. The cage is placed under a sterile hood/changing station.
2. A second empty cage is placed next to the cage. Individual mice are lifted by the tail and placed on the grid.
3. They are observed with regard to their tail and walking abilities (see above and Attachment A).
4. Further, note is taken of general conditions:
 - Reaction to stimulus reaction to hand in the cage
 - Mouse Grimace Scale (MCS) see attachment B
 - Injection site/intervention site check for inflammation/ulceration
 - Respiration check frequency
 - Feeding/drinking check water bottle and chow levels
 - Feces check consistency
 - Blood in cage check cage walls and bedding

For animals presenting not normal for any of these conditions additional scoring is performed according to Attachment B

5. The weight is measured and recorded. Euthanize immediately if weight loss exceeds 25% of weight at onset or when weight loss is 20% - 25% for more than 3 days.
6. The animal is placed in the new cage. The next animal is scored.

The following rules apply for the different types of EAE:

EAE and adoptive transfer EAE

- After induction of EAE, the mice have to be **observed daily** for disability and availability of chow/liquid food and water.
- After onset of EAE symptoms they need to be scored at least **every second day**.
- Above a clinical score of ≥ 3 the mice are scored **daily** until remission below score 3.

oDTR/Diphtheria toxin


- Until d25 score every 4-5 days
- From d25 score every 2 days
- With first symptoms score daily

spontaneous EAE models (BCR/TCR transgenics)

- from three weeks of age score every 3-4 days
- with first symptoms score **every second day**
- Above a clinical score of ≥ 3 the mice are scored **daily** until remission below score 3.

Factors influencing outcome:

The scoring should be performed at the same time of the day

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 4 of 8
Date: 20.09.2018	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-F-EN Version: F

Analysis:

Describe how the data is calculated, how the outcome is interpreted

Documentation:


The scoring needs to be documented by the individual investigator, stored as Excel file in the PROJECT folder of the LTK server and must be available in case of inspection.

Problem management:

If anything unusual is observed regarding the animals, the lab head or a vet have to be informed.

Literatur:

1. Locatelli et al. 2012
2. Standard Operating Procedure Scoring of EAE of the Canton Zurich from 9. Juli 2004
3. Palle et al. 2015

 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 5 of 8
	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-F-EN Version: F

Attachment A

1. Clinical scoring and observation

Score	Clinics	Assessment	Action
0	No detectable signs of EAE		
0.5	Distal limp tail		
1.0	Complete limp tail		
1.5	Limp tail and hind limb weakness	Occasional grid test positive	Provide chow and water on cage floor
2.0	Unilateral partial hind limb paralysis	One leg consistently falls through the grid (cage test)	
2.5	bilateral partial hind limb paralysis	Cage grid test bilaterally positive	
3.0	Complete bilateral hind limb paralysis		euthanize if persistent >7 days
3.5	Complete bilateral hind limb and partial forelimb paralysis		euthanize if persistent >3 days
4.0	Moribund (mouse completely paralyzed)		euthanize immediately
5.0	dead		

2. Measure weight, euthanize immediately if weight loss exceeds 25% of weight at onset or when weight loss is 20% - 25% for more than 3 days.

3. The scoring is documented and stored as xlsx file in the PROJECT folder of LTK Server

4. Check the animals also with each scoring for the following parameters:

- Reaction to stimulus
- Grimace scale
- Injection site/Intervention site
- Respiration
- Fur/body

For animals unusual for any of these conditions, use second page.

EAE and adoptive transfer EAE


- After induction of EAE, the mice have to be **observed daily** for disability and availability of feed and water (For wildtype mice the observation has to start at least 2 days before the known onset of symptoms).
- After onset of EAE symptoms they need to be scored at least **every second day**.
- At clinical score of ≥ 3 the mice are scored **daily** until remission below score 3.

oDTR/Diphtheria toxin/cuprizone

- Until d25 score every 4-5 days
- From d25 score every 2 days
- With first symptoms score daily

spontaneous EAE models (BCR/TCR transgenics)

- from three weeks of age **score every 3-4 days**
- with first symptoms score **every second day**
- At clinical score of ≥ 3 the mice are scored **daily** until remission below score 3.

 University of Zurich Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 6 of 8
Date: 20.09.2018	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-F-EN Version: F

Attachment B

Animals deviating from symptoms described in Attachment A (if necessary use additional pages)


Score these animals daily! Inform the lab head!

1. Parameters

- Reaction to stimulus reaction to hand in the cage
- Mouse Grimace Scale (MCS) see attachment B
- Injection site/intervention site check for inflammation/ulceration/necrosis
- Respiration check frequency
- Fur/body check coat appearance and posture

2. Observation and Action

Score	Stimulus reaction	Grimace Scale	Injection site	Respiration	Fur/body	Action
0	immediate movement	0	normal	normal	normal	none
1	normal but after many stimuli	1	inflamed	increased	dull, unkempt coat	Euthanize when lasting 5 days
2	slow movement after many stimuli	2	ulcerating	heavy	pilo erection	Euthanize when lasting 1 day
3	no reaction after stimuli	-	necrosis	very heavy/ dyspnea	hunched posture, sunken flanks, loss of skin turgor	euthanize


 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 7 of 8
	Date: 20.09.2018	Scoring of Mice after EAE Induction EAE Scoring

Scientist: _____

Start Date: _____

License ID: _____

Date	Cage Nr.	ID	Stimulus Reaction	Grimace Scale	Injection site	Respiration	Fur/body	Weight (g/% start)	Intervention	Signature

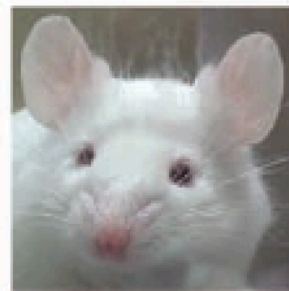
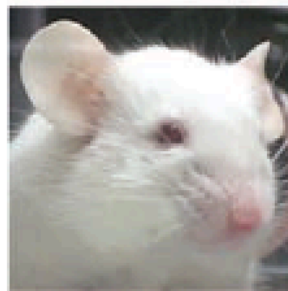
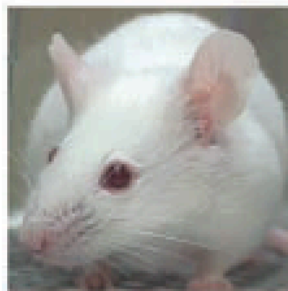
 University of Zurich ^{UZH} Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 8 of 8
Date: 20.09.2018	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-F-EN Version: F

Attachment B
Minimal Mouse grimace scale (MGS)

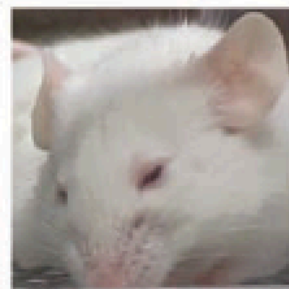
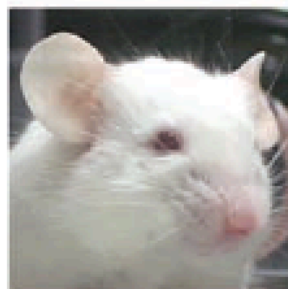
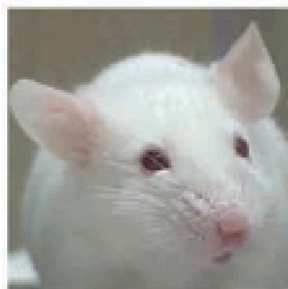
0

1

2



Nose bulge



Cheek bulge