



Date 20160616

Name JR/AL

Standard Operating Procedure Tumor Cell Injections

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Intravenous tumor cell injection

1. Gently warm mice using an IR-lamp to make the veins become more visible
2. Place the mouse in a restraining tube
3. Inject the tumor cells in a maximum of 200ul liquid (e.g. PBS) slowly into the tail vein using an insulin syringe
4. Starting with the injection of live tumor cells, mice should be monitored according to the guideline and respective score sheet "EBV Vaccination Tumor Challenge".

Subcutaneous tumor cell injection

1. Anesthetize mice using an Isoflurane-evaporation chamber according to the SOP "Anesthesia" being part of the 209/2014 license.
2. Remove mice from the chamber and remove its fur on the site of injection to simplify measurements of tumor sizes afterwards as well. Gently inject the tumor cells in up to 100ul of liquid into the neck skin or into lateral flanks using an insulin syringe
3. Starting with the injection of live tumor cells, mice should be monitored according to the guideline and respective score sheet "EBV Vaccination Tumor Challenge".

209/2014 Health scoring: EBV Vaccination Tumor Challenge

After the injection of live tumor cells, mice should be weight measured and monitored for general and experiment-specific symptoms three times per week at regular intervals. Health scoring sheets "EBV Vaccination Tumor Challenge" will be filled according to the guidelines attached to the score sheet. Symptoms will be graded by individual scores and cumulative scores will be generated. Animal measures will be executed according to the scoring system/guideline. When no symptoms are present, one entry (date, score, signature) for the whole group/cage is sufficient. When symptoms are apparent, one entry should be made for each affected mouse. Score sheets can be digitalized and saved on the group's server to be accessible at any times.

Parameter	Description	Score	Measure
Fur	no symptoms	0	
	not well groomed, rough hair coat	1	
	ungroomed hair	2	
Posture, general appearance	no symptoms	0	
	slightly hunched posture	1	
	clear hunchback	10	Monitor everyday; provide gel food; euthanize in case the symptom persists for more than 2 days
	severe hunchback and paleness	38	Immediate euthanasia free of pain
Activity and Behaviour	no symptoms	0	
	slightly reduced activity	1	
	reduced activity	10	Monitor everyday; provide gel food; consider single housing if it's beneficial for the mouse; euthanize in case the symptom persists for more than 2 days
	vocalization	38	Immediate euthanasia free of pain
	abnormal breathing (e.g. breathe heavily, gasping)	38	Immediate euthanasia free of pain
	complete inactivity and isolation	38	Immediate euthanasia free of pain
	missing escape reflex upon touch	39	Immediate euthanasia free of pain
Eyes, nose	normal	0	
	eyes squint	10	Monitor everyday; provide gel food; euthanize in case the symptom persists for more than 2 days
	eyes squint and bulged nose	18	Monitor everyday; provide gel food; euthanize in case the symptom persists for more than 1 day
Tumor Assessment	no tumor or tumor size <10mm	0	
	subcutaneous tumor ≥10mm	5	monitor everyday, provide gel food
	ulceration or other wound formations	38	Immediate euthanasia free of pain
	subcutaneous tumor ≥15mm	39	Immediate euthanasia free of pain
Weight loss	≤10%	0	
	11-15%	5	
	16-20%	15	Monitor everyday; provide gel food; euthanize in case the symptom persists for more than 2 days
	≥ 20%	38	Immediate euthanasia free of pain
Cumulative score		0-9	monitor everyday, provide gel food
		10-17	Monitor everyday; provide gel food; euthanize in case the symptom persists for more than 2 days
		18-37	Monitor everyday; provide gel food; euthanize in case the symptom persists for more than 1 day
		≥38	Immediate euthanasia free of pain