Protocol #	Tissue-001

Title	**ATP determination**
Lit. Ref	
Lab. Ref	(Roche ATP assay kit) — *Latest Update* BT *Date* 21/10/13
Date	10/16/13
Goal	Assay the ATP level in tissue slice
Materials	• **safelock vials** • **minibead-beater** • **Repetitive pipet with 50 µl tip** • **White 96-wells plate** • **Syrnegy HT plate reader**
Solutions	• **SONOP (Sonification Solution), Ethanol (70% v/v) containing 2 mM EDTA (M=372.24 g/mol) with pH=10.9**. *For 500 ml : Dissolve 0.372 g EDTA in ± 100 ml of mQ-water, adjust PH with 5M NaOH to PH=10.9, add 30mL MQ-water and 370 ml Ethanol (96%).* • **100mM Tris-HCl, 2mM EDTA buffer (pH 7.6-8.0)** *For 500 ml: Dissolve 6.0 g Tris (M=121.14) (Tris(hydroxymethyl)amniophen; Merck) and 0.37 g EDTA (Triplex III; M=372.24) in ± 300 ml MQ-water, adjust pH with 6N HCl and fill up to 500 ml total volume with MQ-water* • **ATP Bioluminescence assay kit Roche.** Contents: Luciferase reagent lyophilized (white cap) *(Dissolve lyophilized luciferase in exactly 10.0 ml MQ-water and mix by swinging. Do not vortex)* ATP-standard ± 10 mg lyophilized (red cap) *(Dissolve the ATP-standard from the kit to **exactly** 10 mg/ml (= 16.5mM) with ultra pure water)*

Protocol

After the incubation put 1 slice in 1 ml SONOP in a safelock vial, 1 cup of minibead and snap frozen in liquid N_2.

1. Homogenize the sample with minibead-beater for 45 seconds. Keep the samples on ice.
2. Centrifuge homogenate 5 minutes at 13,000 rpm. Transfer the supernatant into a new tube and keep on ice, the tube with precipitate is dried at 37°C (1 day) or room temperature (3 days) for protein measurement.
3. Make a calibration curve:

Dilution A, B and C are only to prepare the calibration curve, they are not going to be measured. Calibration samples (a,b,c, Cal1-5) should not be stored to reuse

Dilution	Amount (µl)	Tris/EDTA Buffer (µl)	Conc. (M)
A	10µl ATP-standard	***90***	1.65×10^{-3}
B	50µl [A]	450	1.65×10^{-4}
C	50µl [B]	450	1.65×10^{-5}
Cal 1	50µl [C]	450	1.65×10^{-6}
Cal 2	***100µl [1]!***	***400***	3.30×10^{-7}
Cal 3	50µl [1]!	450	1.65×10^{-7}
Cal *4*	***100µl [3]!***	***400***	3.30×10^{-8}
Cal 5	50µl [3]!	450	1.65×10^{-8}

4. Pipet 5 µl supernatant + 45 µl Tris/EDTA buffer into white 96-wells plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank1	Blank1	A7	A7	A15	A15	A23	A23	A31	A31	Cal1	Cal1
B	Positive	Positive	A8	A8	A16	A16	A24	A24	A32	A32	Cal2	Cal2
C	A1	A1	A9	A9	A17	A17	A25	A25	A33	A33	Cal3	Cal3
D	A2	A2	A10	A10	A18	A18	A26	A26	A34	A34	Cal4	Cal4
E	A3	A3	A11	A11	A19	A19	A27	A27	A35	A35	Cal5	Cal5
F	A4	A4	A12	A12	A20	A20	A28	A28	A36	A36	Blank2	Blank2
G	A5	A5	A13	A13	A21	A21	A29	A29	A37	A37	A39	A39
H	A6	A6	A14	A14	A22	A22	A30	A30	A38	A38		

5. Always include a positive control (stored at –80˚C). Prepare in the same way like the homogenates
6. Pipette 50 µl diluted calibration curve in a white 96-wells plate (at 4˚C)
7. Add to every well 50 µl luciferase (4˚C) by using repetitive pipet with 5mL combi tip (can be attached with 100uL yellow tip)
8. Measure plate after 0, 5 en 10 minutes with the luminometer (follow the SynergyHT protocol)

Remarks	**a) Important!: The ATP in the slices may be breaking down by present enzyms, therefore store samples at -80°C and keep everything at 4˚C during determination**