SUPPLEMENTARY INFORMATION

A Guide to *Ex Vivo* Biodistribution Studies with Radiotracers in Rodent Models

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Table S1. An example of syringe preparation form.

Date: 23/10/2024				**Radiosynthesis batch no.:** 20241023-DT1			
Researcher: Surachet Imlimthan				**Tracer/Compound:** [^{68}Ga]Ga-DOTATATE			
Activity concentration: 20 MBq mL^{-1}				**Formulation/dose:** 100 μL in 0.9% isotonic saline			
Syringe ID	**Activity (Before injection, MBq or μCi)**	**Time**	**Syringe weight (Before injection, g or mg)**	**Residual activity (After injection, MBq or μCi)**	**Time**	**Syringe weight (After injection, g or mg)**	**Remark***
1	1.715	9:31	1.9804	0.018	11:48	1.8806	
2	1.721	9:32	1.9738	0.021	11:49	1.9229	
3	1.718	9:33	1.9849	0.020	11:50	1.8816	
4	1.744	9:34	1.9876	0.010	11:51	1.8759	Standard

**Corresponding animal ID and details can be added to the remark.*

Table S2. An example of animal injection sheet.

Date: 23/10/2024				**Animal license no.:** XXXXXX			
Researcher: Surachet Imlimthan				**Tracer/Compound:** [^{68}Ga]Ga-DOTATATE (Batch no.: 20241023-DT1)			
Formulation: in 0.9% isotonic saline, 5% EtOH				**Injected volume:** approx. 100 µL per dose			
Syringe ID	**Animal ID**	**Tail/ear marking**	**Time point**	**Injection time**	**End point**	**Body weight (g)**	**Tumor measurement (W and L in mm)***
1	M1	●	15 min	9:15	9:30	20.1280	W=3.07, L=3.08
2	M2	●	30 min	9:30	10:00	19.8572	W=3.94, L=4.11
3	M3	●	60 min	10:15	11:15	20.0017	W=3.70, L=4.50

**W for width (the smallest dimension) and L for length (the largest dimension) measured by Vernier calipers. Tumor volume = 0.5×L×W^2*

Table S3. An example of a general tissue list for *ex vivo* biodistribution studies.

Entry	Organ
Before opening the abdominal cavity	
1	Blood (cardiac puncture)
2	Tumor
After opening the thoracic and abdominal cavity (collecting from top to bottom)	
3	Heart
4	Lung
5	Gallbladder
6	Liver
7	Spleen
8	Pancreas
9	Stomach
10	Small intestines
11	Large intestines
12	Adrenal gland
13	Kidneys
14	Fat
15	Other internal organs of interest
16	Urine (from the bladder or CO_2 chamber)
17	Muscle
18	Tibia
19	Tail (injection site)
Head-and-neck region	
20	Skin (ear flap)
21	Occipital bone
22	Brain

Table S4. Acquired information and input flow of the Microsoft Excel-based biodistribution calculator.

Entry	Category	Item	Input location	Remark
1	General information	Name of radiotracer	C2	
2		Start of the counting time	C3	Format: DD.MM.YYYY HH:MM Note: All input values of activity are decay corrected to this time point.
3		Dose calibrator background (MBq)	C4	
4		Radionuclide	C5	Selection from a drop-down menu. Data provided are from the National Nuclear Decay Center, https://www.nndc.bnl.gov/nudat3/
5		Number of animals (n)	C8	The Excel sheet contains sufficient space for a group of n = 15 animals. If additional space is required new rows & columns can be inserted
6	Animal, syringe, and injection details	Animal ID	B13 – B27	
7		Whole-body weight (g)	C13 – C27	
8		Syringe ID	D13 – D27	
9		Full syringe weight (g)	E13 – E27	Before animal injection
10		Empty syringe weight (g)	F13 – F27	After animal injection
11		Full syringe radioactivity (MBq)	H13 – H27	Measured activity in the syringe before injection. Note the activity should be input without correction for background activity which is performed separately. Alternatively, if a manual correction for background is performed, the cell C4 should be zero.

12		Full syringe date and time	I13 – I27	Recorded time of the dose calibrator assay of the full syringe. Format: DD.MM.YYYY HH:MM
13		Animal injection date and time	J13 – J27	Format: DD.MM.YYYY HH:MM
14		Empty/residual syringe radioactivity (MBq)	K13 – K27	Measured residual activity in the syringe after injection. See entry 11 for the note about background correction.
15		Empty/residual syringe date and time	From L13	Recorded time of the dose calibrator assay of the empty syringe. Format: DD.MM.YYYY HH:MM
16	Standard syringe details	Full standard syringe weight (g)	E28	Full syringe mass recorded before injection into a falcon tube containing water.
17		Empty standard syringe weight (g)	F28	Empty / residual syringe mass recorded after injection into a falcon tube containing water.
18		Full standard syringe radioactivity (MBq)	H28	Measured activity in the syringe before injection. See entry 11 for the note about background correction.
19		Full standard syringe date and time	I28	Format: DD.MM.YYYY HH:MM
20		Injection time (into dilution tube)	J28	Format: DD.MM.YYYY HH:MM
21		Empty/residual standard syringe radioactivity (MBq)	K28	Measured residual activity in the syringe after injection into the falcon tube containing water. See entry 11 for the note about background correction.
22		Empty/residual standard syringe date and time	L28	Format: DD.MM.YYYY HH:MM
23	Standard syringe	Empty 15-mL conical centrifuge tube weight (g)	C32	

24	dilution details	Tube + 9.9 mL of water weight (g)	C33	Note: the precise volume of water used for the dilution is not important. The critical factor is to know the dilution mass obtained and from experiences, approximately 10 mL of water is sufficient to avoid saturating the gamma counter with the prepared standard count tubes.
25		Tube + water + standard weight (g)	C34	After injection of the standard syringe into the tube containing water.
26	Background counts	Blank counts (CPM)	From C41 to C44	Note: Use non-decay corrected CPM (n = 4) from the gamma counter. Average background counts are calculated in cell C45
27	Standard syringe calibration	Empty gamma counting tube weight (g)	From C51 to C54	Use CPM data from the gamma counter that is decay-corrected to the start of gamma count measurement. Standards are counted in quadruplicate (samples A – D). Standards are used to calculate a value of CPM g^{-1} of injectate, and also a calibration factor of CPM/MBq. In the event that no radiotracer sticks to the syringe, the CPM g^{-1} values can be used but a full correction, accounting for residual activity in the individual syringes is given by using the CPM MBq^{-1} correction factor.
28		Full gamma counting tube weight (g)	From D51 to D54	
29		Normalized standard counts (CPM)	From F51 to F54	
		Counting standards	Rows 71 – 74	Columns B – K
30	Gamma counting results of tissues	Counting blanks	Rows 75 - 78	Data are recorded but inserted into a separate part of the Excel spreadsheet. See Entries 26–29 (above)
31		Animal ID	Rows B79 – B318	Animal assignment. All tissues collected for one individual animal are counted sequentially. The same order of tissues is used for each animal.

32		Sample assignment	Rows C79 – C318	Reference: Table S3. This should be amended based on the user's preference for the counting order of tissues.
33		Empty organ tube weight (g)	Rows D79 – D318	Tube + cap weight, empty.
34		Organ-filled tube weight (g)	Rows E79 – E318	Tube + cap + tissue sample weight, full. The sample mass for each tissue is calculated in cells F79 – F318.
35		Normalized sample counts (CPM)	Rows G79 – G318	Note: Use CPM values decay-corrected to the start of the gamma count measurement. Rows H79 – H318 calculated the background corrected CPM g^{-1} values after subtracting the average background activity in CPM in cell C45, then dividing by the sample mass (cells F79 – F318). Sample activity in units of MBq g^{-1} are calculated in cells I79 – I318. Tissue uptake in %ID g^{-1} is calculated for each individual sample in cells J79 – J318. SUV (by weight, in g) is calculated in cells K79 – K318.

Note that the Excel spreadsheet provided four sections of output with the associated bar charts shown underneath each table.

Blue table (starting in the top left cell P10) gives the biodistribution data in units of %ID g^{-1}.

Green table (starting in the top left cell P48) gives the biodistribution data in units of SUV (by weight in grams).

Red table (starting in the top left cell AI10) gives the tumor-to-tissue contrast ratios calculated as dependent data sets within each individual animal, based on the biodistribution data in units of %ID g^{-1}.

Purple table (starting in the top left cell AI48) gives the tumor-to-tissue contrast ratios calculated as dependent data sets within each individual animal, based on the SUV data.

Calculation of tissue uptake in units of %ID g^{-1} and SUV

Calculated injected activity at injection time (MBq)

= [(full syringe activity – dose calibrator BG) × Exp((–λ)(injection time – syringe preparation time) × 24h)] – [(residual syringe activity – dose calibrator BG) × Exp((–λ)(injection time – residual syringe time) × 24h)] (Eq.1)

Where: λ = decay constant and Δ time = day

Calculated injected activity (MBq)

= [Calculated injected activity at injection time (MBq, or Eq.1)] × Exp[(–λ)(start of counting time – injection time) × 24h] (Eq.2)

Background (BG) corrected normalized counts per sample mass (CPM g^{-1})

= [Normalized sample counts (CPM) – Average non-decay corrected BG counts (CPM)]/[Sample mass (g)] (Eq.3)

Standard (Std) calibration factor (CPM MBq^{-1})

= [Total normalized BG corrected counts per sample mass (CPM g^{-1})]/[Calculated injected activity (MBq)/Calculated injected mass (g)] (Eq.4)

Calibrated sample activity/mass (MBq g^{-1}) = (Eq.3)/(Eq.4)

= [BG corrected normalized counts per sample mass (CPM g^{-1})]/[Std calibration factor (CPM MBq^{-1})] (Eq.5)

%ID g^{-1} = [(Eq.5)×100]/(Eq.2)

= [Calibrated sample activity per sample mass (MBq g^{-1})×100]/[Calculated injected activity (MBq)] (Eq.6)

SUV = [%ID g^{-1} (or Eq.6)]/[whole-body weight (g)/100] (Eq.7)