

Materials List for:

Radionuclide-fluorescence Reporter Gene Imaging to Track Tumor Progression in Rodent Tumor Models

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Materials

Name	Company	Catalog Number	Comments
Step 1) Engineering and characterization of cancer cells to express the radionuclide-fluorescence fusion reporter NIS-FP.			
2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-Bi-1H-benzimidazole	Thermo Scientific	H3570	Trivial name: Hoechst 33342; CAS number: 23491-52-3; Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water.
4T1 murine breast cancer cell line	ATCC	CRI-2539	for details see ATCC website
Automatic Cell Counter, e.g. CASYCounter	Roche Diagnostics GmbH	5651697001	CASY Model TT Cell Counter and Analyzer
CASYclean Cleaning Reagent	Sedna Scientific	2501036	
CASYton Isotonic Diluent	Sedna Scientific	2501037	
Confocal Fluorescence Microscope, e.g. Leica TCS SP5	Leica, Wetzlar, Germany		Equipped with Plan-Neofluor 25×0.5NA and Plan-Apochromat 63×1.4NA oil UV objectives and Diode (405 nm), Argon-ion (458, 477, 488, 496, 514 nm) and HeNe (543 and 633 nm) lasers; A Leica LAS AF Lite Software 4.0.11706 (Leica Microsystems CMS GmbH) was used for image acquisition and analysis
Cover slips No. 1.5 thickness	VWR International	631-0150	
Dabco	Sigma	290734	Stock 125 mg/mL
DMEM	Sigma	D5546	Supplement with 10 % (v/v) FBS and L-glutamine (2 mM) to make up the optimal growth medium for MDA-MB-231 cells.
FACS sorter, e.g. BD FACSAria III	BD Biosciences		Equipped with a BD FACS DIVA Software, a 6 Laser System (375/405/488/561/633 nm lasers) - cells sorted with a 100 µm nozzle under 20 psi flow pressure, window extension of 2.0 µm, 2.0 Neutral Density Filter and 3 kV plate voltage
Fetal Bovine Serum (FBS)	Sigma	F9665	Heat inactivated at 56 °C for 30 min
Automated Gamma Counter, e.g. 1282 Compugamma	LKB Wallac Laboratory		^{99m} Tc-pertechnetate energy window 110-155 keV 18F energy window 175-220 keV

Hoechst 33342 solution	Life Technologies	H1399	1-3 µg/mL; DAPI (from various suppliers) can be used instead.
L-glutamine	Sigma	G7513	Solution 200 mM concentrated, sterile-filtered
Linear polyethylenimine (PEI)	Polyscience	23966-2	Linear, 25 kDa; transfection reagent for 293T cell line.
MDA-MB-231 human breast cancer cells	ATCC	HTB-26	for details see ATCC website
Mowiol 4-88	Sigma	81381	
pLNT SFFV NIS-mEGFP	request from our lab	n/a	For details (generation and maps) see Supplementary Information
pLNT SFFV NIS-mCherry	request from our lab	n/a	For details (generation and maps) see Supplementary Information
pMD2.G	Addgene	#12259	plasmids encoding for the VSV-G envelope
pRRE	Addgene	#12251	packaging plasmid
pRSV-Rev	Addgene	#12253	packaging plasmid
Paraformaldehyde solution 4 % (w/v) in PBS	Santa Cruz Biotechnology	sc-281692	
Penicillin-Streptomycin	Sigma	P43330	Containing penicillin (10,000 units/mL) and streptomycin (10 mg/mL), sterile-filtered
Phosphate Buffered Saline (PBS)	Sigma	D8537	pH 7.4, sterile-filtered and without calcium chloride and magnesium chloride
Poly(vinyl alcohol - vinyl acetate)	Polysciences	17951	Trivial name: Mowiol 4-88; CAS number: 9002-89-5
Puromycin dihydrochloride	Sigma	P8833	From <i>Streptomyces alboniger</i> , reconstituted in sterile water
Benchtop centrifuge, e.g. Rotina 380 R Benchtop centrifuge	Hettich Lab Technology		
RPMI 1640	Sigma	R0883	Supplement with 10% (v/v) FBS and L-glutamine (2mM) to make up the optimal growth medium for 4T1 cells.
SFCA Syringe filter 0.45 µm	Corning		
Syringes 10 mL	BD Emerald		Disposable non-sterile syringes
Tissue culture fluorescence microscope, e.g. EVOS-FL	Life Technologies		Cell Imaging System equipped with a 10× objective (PlanFL PH2, 10×/0.25, ∞/1.2) and a colour camera
Trypsin-EDTA solution 10X	Sigma	59418C	(0.5 % (w/v) trypsin, 0.2 % (w/v) EDTA) gamma irradiated by SERTAIN process and without phenol red
Wheat Germ Agglutinin Alexa Fluor 633 Conjugate	Life Technologies	W21404	Used at 1:1000 (2 µg/mL) for cell immunofluorescence
Step 2) Establishment of <i>in vivo</i> tumor models.			
Digital caliper	World Precision Instruments	501601	
Isoflurane 1000 mg/g	Isocare		For inhalation
Fluorescence Torch, e.g. NightSea Fluorescence Torch DFP-1	Electron Microscopy Sciences	SFA-LFS-RBS/GR	Equipped with GFP and RFP emission filters and NightSea filter goggles (DFP-1)
Syringes 0.3 mL U-100 insulin	Terumo		29G × 1/2" - 0.33 × 12 mm

Standard materials/equipment for aseptic technique and animal maintenance			
Step 3) Production of [¹⁸F]BF₄⁻ using an automated radiotracer synthesis platform.			
15-crown-5	Sigma-Aldrich	188832	CAS 33100-27-5
Acetonitrile (anhydrous)	Acros Organics	326811000	
Boron trifluoride diethyl etherate	Sigma-Aldrich	216607	BF ₃ .OEt ₂ , purified by redistillation, ≥46.5 % BF ₃ basis. CAS 109-63-7
Automated Radiotracer Synthesis (ARS) platform, e.g. FASTLab	GE Healthcare		
Disposable cassettes for ARS platform, e.g. FASTLab cassettes	GE Healthcare		FASTlab Developer pack
Polygram Alox N/UV254 polyester sheets	Macherey-Nagel	802021	RadioTLC plates, 40×80 mm
Strong anion exchange cartridge, e.g. Sep-Pak Accell Plus QMA Plus Light	Waters	WAT023525	Condition with 1M NaCl (10 mL) and H ₂ O (10 mL)
Alumina neutral cartridge, e.g. Sep-Pak Alumina N Plus Light	Waters	WAT023561	Condition with H ₂ O (10 mL), acetone (10 mL) and air (20 mL)
Water for injection USP	GE Healthcare		
Nitrogen filter	Millipore	SE2M049I05	Sterile 0.2 μm FG Millex 13 mm
Step 4) In vivo imaging of NIS-FP expressing cells by nanoPET/CT.			
Isoflurane 1000 mg/g	Isocare		For inhalation
Preclinical PET/CT multimodal imaging instrument, e.g. nanoScan PET/CT	Mediso Medical Imaging System, Budapest, Hungary		
Fluorescence Torch, e.g. NightSea Fluorescence Torch DFP-1	Electron Microscopy Sciences	SFA-LFS-RBS/GR	Equipped with GFP and RFP emission filters and NightSea filter goggles (DFP-1)
Rodent anesthesia induction chamber	Vet-Tech	AN010R	With three-way valves (x2), tube mount connector for inlet, PVC tubing for gas inlet (2 m) and 22 mm scavenging tube (2 m)
Rodent anesthesia system	Vet-Tech	AN001B	Including animal face-mask suitably sized for animal of interest and isoflurane vaporizer
Sterile physiological saline	Thermo Scientific Oxoid	BO0334B	
Syringes 0.3 mL U-100 insulin	Terumo		29G × 1/2" - 0.33 × 12 mm, for intravenous injection of radiotracer
Veterinary Scavenger	Vet-Tech	AN200	VetScav filter weighing mechanism - 240 V with automatic temperature compensation and LED system
5) In vivo data analysis.			
Tera-Tomo Monte Carlo based full 3D iterative algorithm	Mediso Medical Imaging System, Budapest, Hungary		
VivoQuant Software	Invicro LLC., Boston, USA		
6) Ex vivo analyses			
2-Methylbutane	Sigma	59070-1L-D	Pre-cooled over liquid nitrogen to freeze OCT-embedded tissues
Bovine Serum Albumin (BSA)	Sigma	85040C	
Cover slips 22×50 mm	VWR International	SMITMCQ211022X50	

Cryostat, e.g. Cryostat MNT	SLEE Medical		two-piece modular histology embedding machine equipped with an embedding module, a tissue storage compartment and a cold plate
Cy5 AffiniPure Goat anti-Rabbit IgG (H+L)	Jackson/Strattech	111-175-144	Used at 1:500 (2 µg/mL)
Dabco	Sigma	290734	Stock 125 mg/mL
Microtome blades, e.g. Feather S35	CellPath		
Fluorescence Microscope (wide-field or confocal), e.g. Nikon Eclipse Ti-E Inverted Fluorescence Microscope	Nikon		Equipped with 10×, 20× (air) and ideally 40× (oil) objectives and lasers/filters or filter cubes, respectively, that are suitable for Hoechst 33342, GFP and Cy5
Automated Gamma Counter, e.g. 1282 Compugamma	LKB Wallac Laboratory		^{99m} Tc-pertechnetate energy window 110-155 keV, 18F energy window 175-220 keV
Hoechst 33342 solution	Life Technologies	H1399	
Fluorescence adapter for dissecting microscope, e.g. NightSea Adapter	Electron Microscopy Sciences	SFA-LFS-RBS/GR	Equipped with GFP and RFP emission filters
O.C.T. compound	VWR international	361603E	
Wax pen, e.g. PAP-PEN	Dako UK Ltd		Wax pen to draw around tissue section to reduce required staining/washing solution volumes
Paraformaldehyde solution 4 % (w/v) in PBS	Santa Cruz Biotechnology	sc-281692	
Rabbit anti-CD31	Abcam	ab28364	Polyclonal anti-mouse used 1:50 (20 µg/mL) for tissues immunofluorescence
Microscope slides, e.g. Superfrost slides	VWR, Lutterworth, UK		
Tris-buffered saline (TBS)	available from various suppliers.		Tris-buffered saline; 150 mM NaCl, 25 mM Tris/HCl at pH 7.4