NATIONAL RESEARCH CENTER "KURCHATOV INSTITUTE"

Laboratory Generator for ^{212}Pb Production

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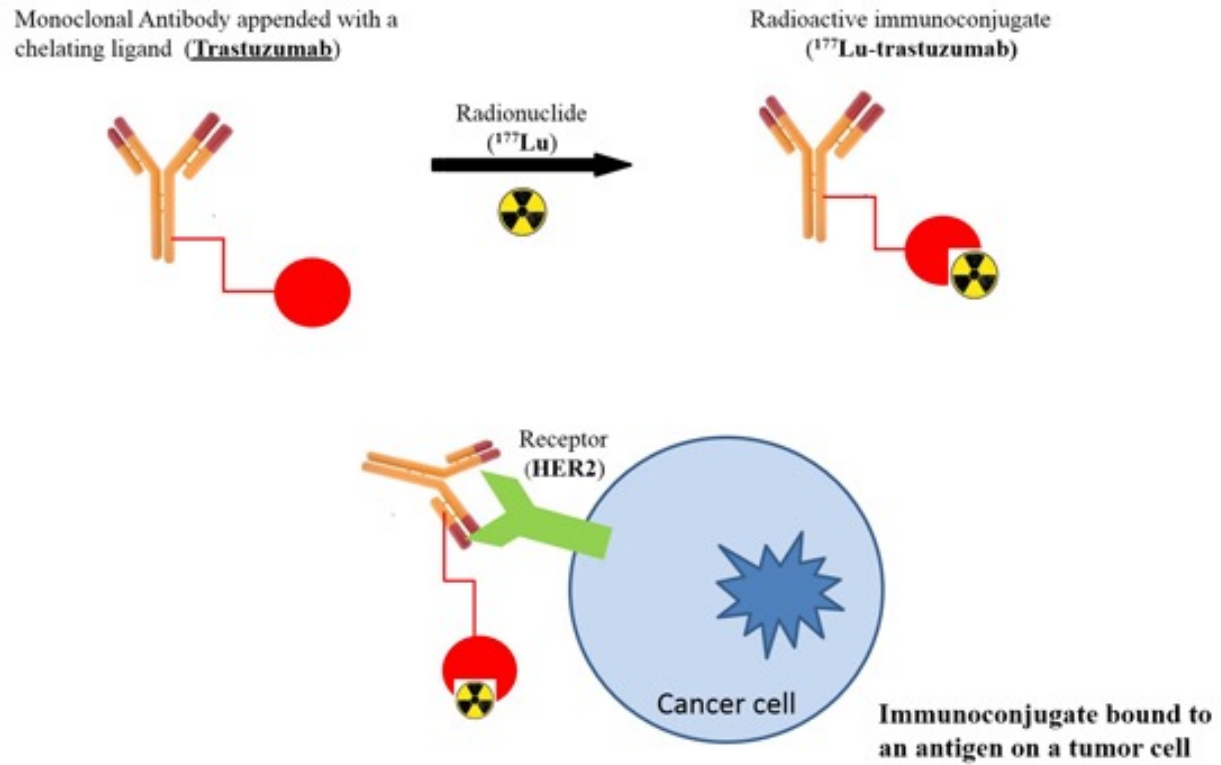
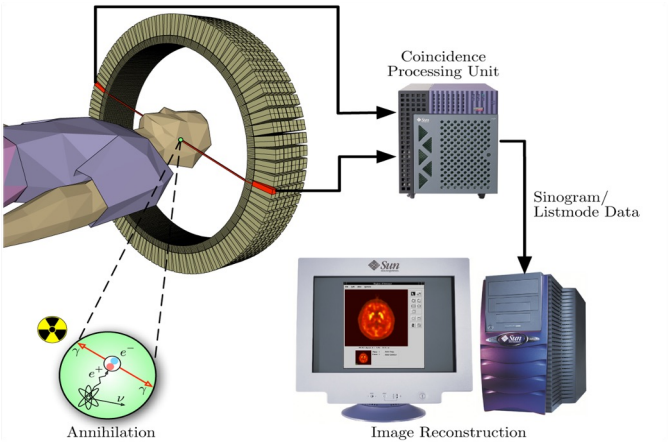
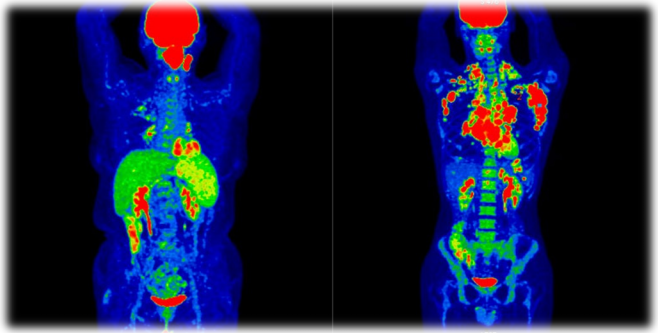
Physical and Chemical Technology Complex

Radionuclide Therapy (Targeted Therapy)

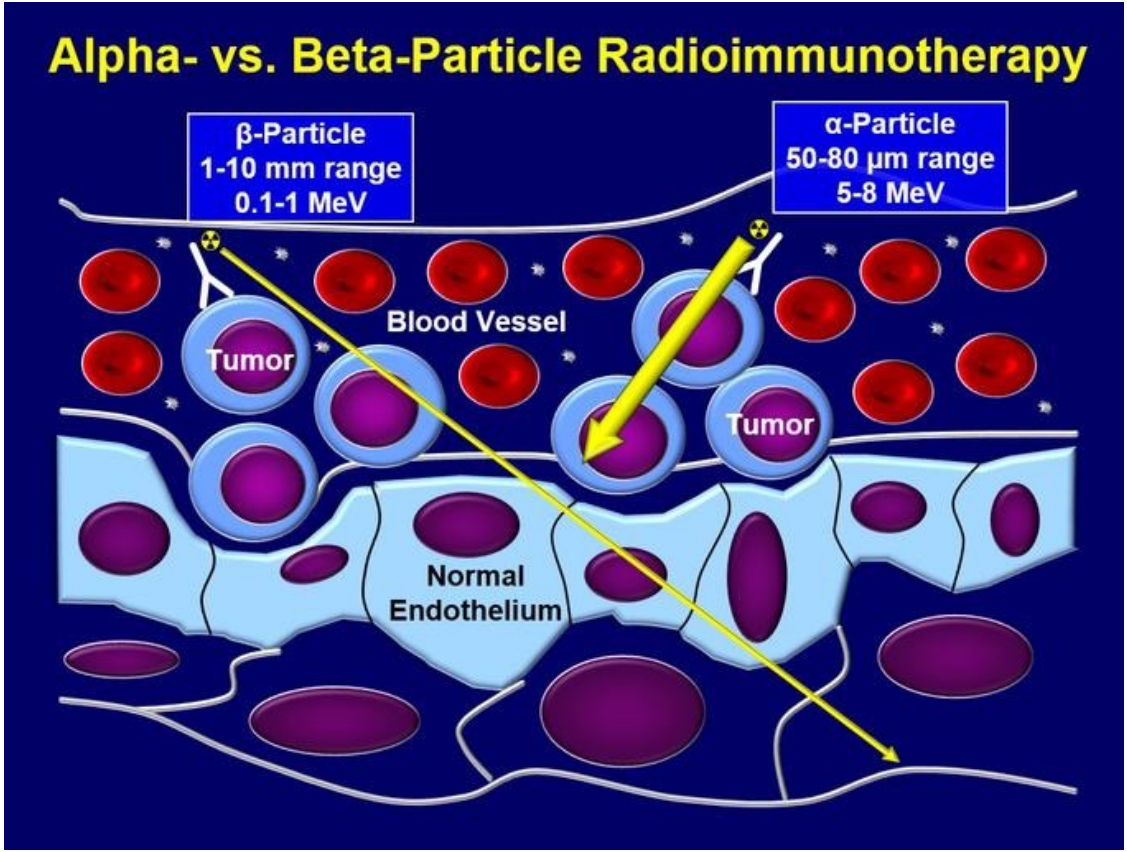


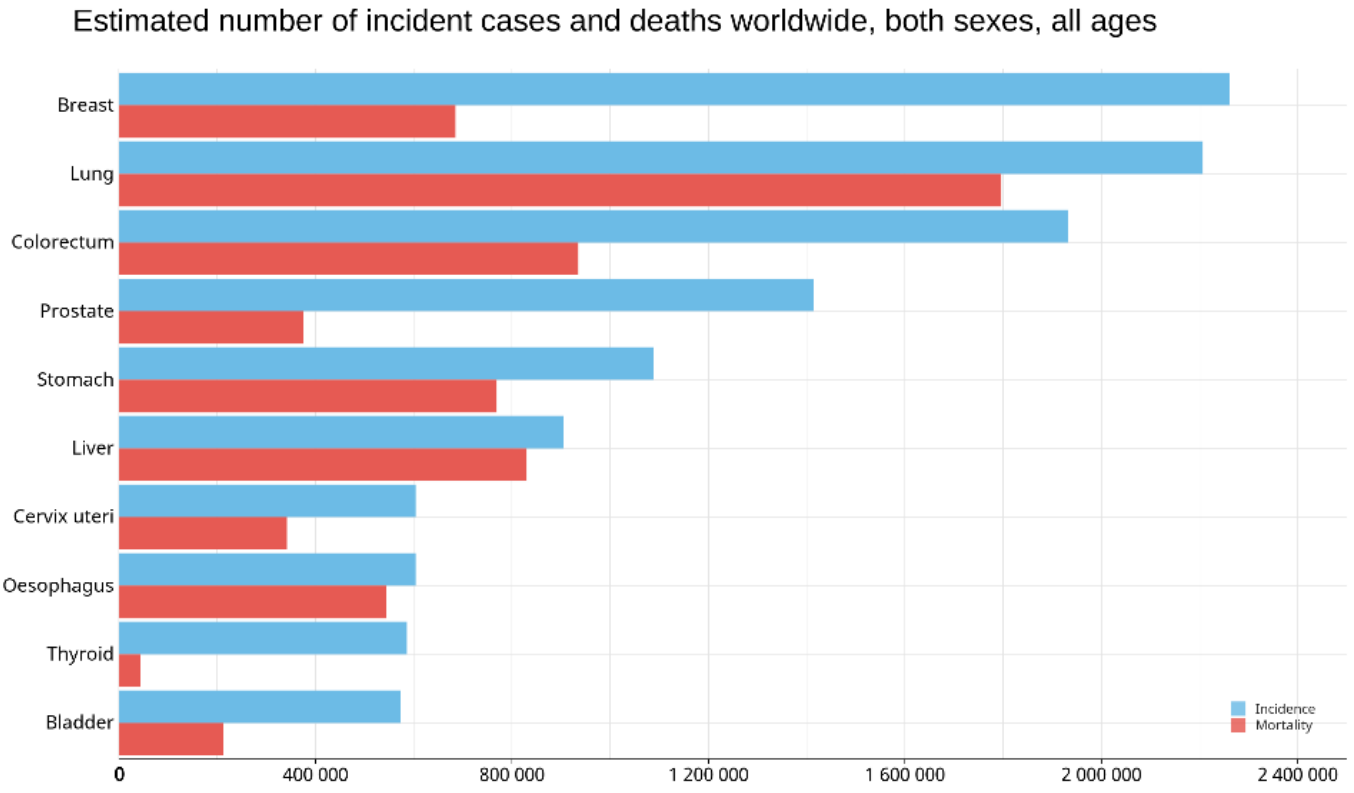
Radionuclide Diagnostics



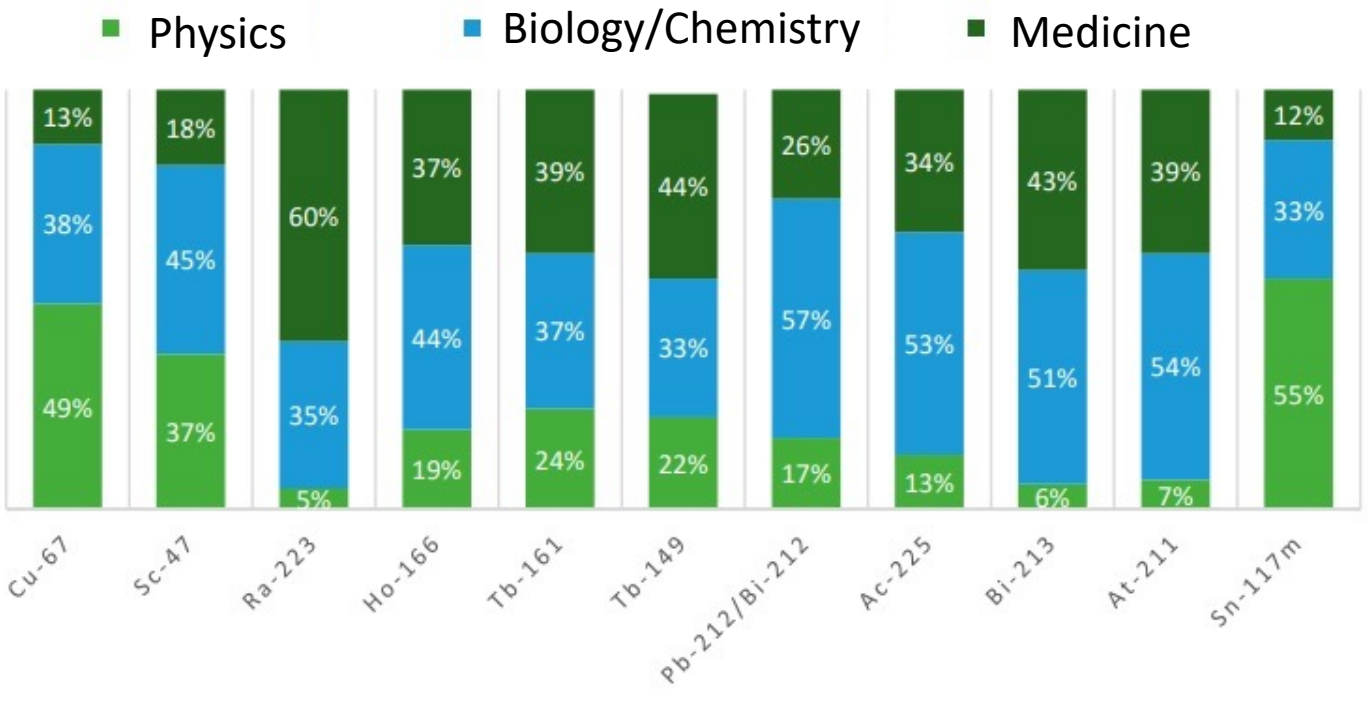


Targeted Therapy

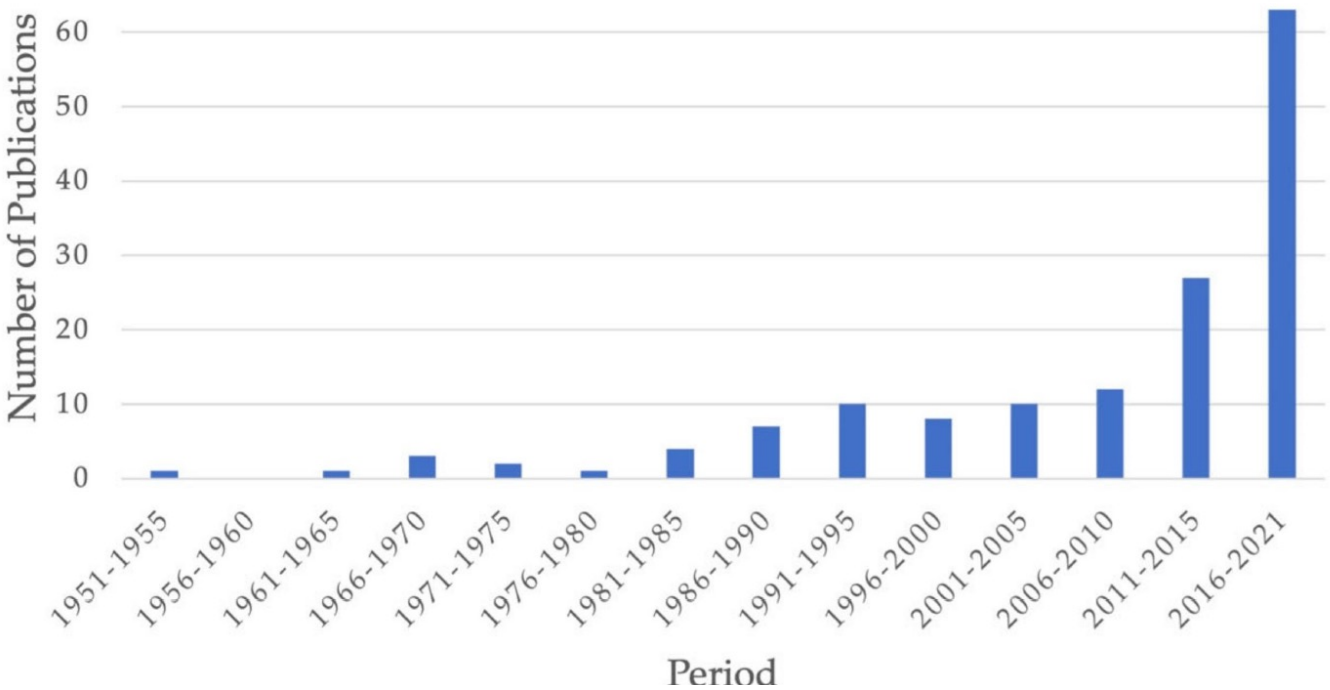




Cancer is currently one of the most common causes of death among the population (10 million deaths in 2020)



Publications on the use of therapeutic radionuclides (2008 - 2018)



Dynamics of publications on the use of lead-212 in nuclear medicine

Goals of Investigation

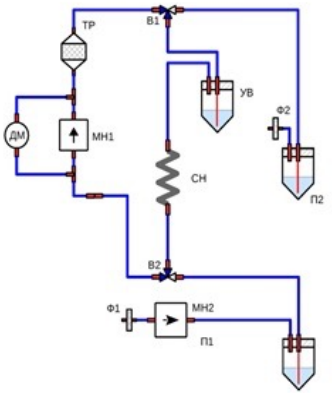
The aim of this work was **to obtain compounds based on ^{212}Pb** for potential radiopharmaceutical applications.

To achieve the goal, the following tasks were solved:

- To create a laboratory ^{212}Pb generator based on the parent ^{228}Th ($T_{1/2}$ = 1.91 y). Confirm the radionuclide purity of the resulting product.
- To carry out the synthesis of compounds for targeted delivery containing accumulated ^{212}Pb as a therapeutic agent.
- Determine the degree of stability of the synthesized compounds in biological relevant media.
- Demonstrate the presence of a cytotoxic effect of the synthesized compound based on ^{212}Pb in *in vitro* experiments.

Plan of Investigation

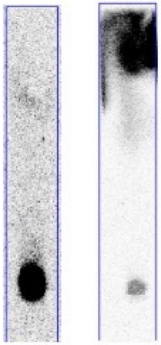
A ^{212}Pb generator



Obtaining of ^{212}Pb

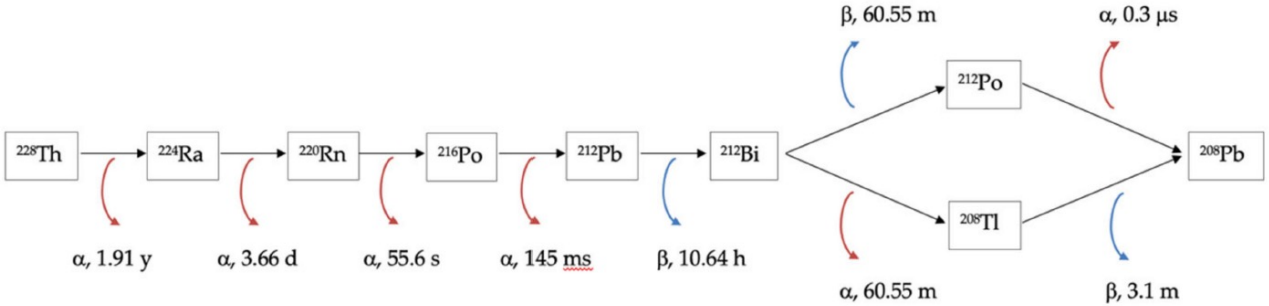
Synthesis

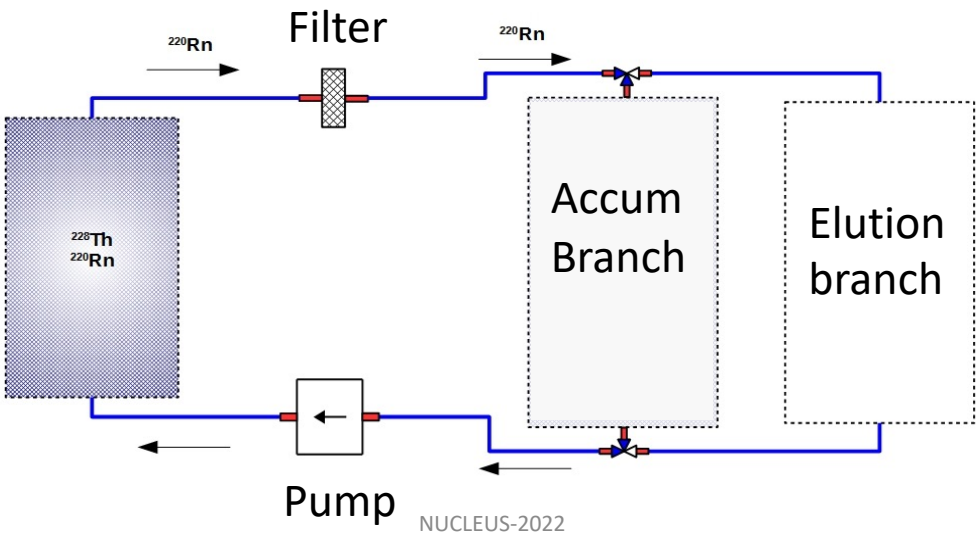
Radiochemical purity



Stability

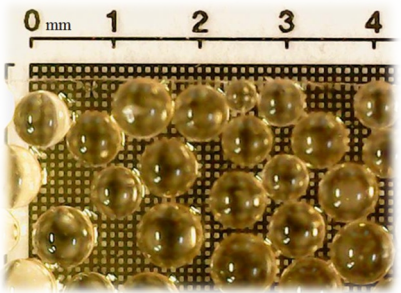
^{212}Pb Obtaining Principle





Generator Source

- $^{232}U/^{233}U$ isolation (50 years) resulted in Thorium source: ^{229}Th – 6,81%; $^{230}Th \cong$ 0,08%; $^{228}Th \cong$ 0,02%; ^{232}Th – 93,11%
- Sorption on strong anion exchange resin in 8 M HNO_3 medium: formation of $Th(NO_3)_5^-$ (2%), $Th(NO_3)_6^{2-}$ (98%)



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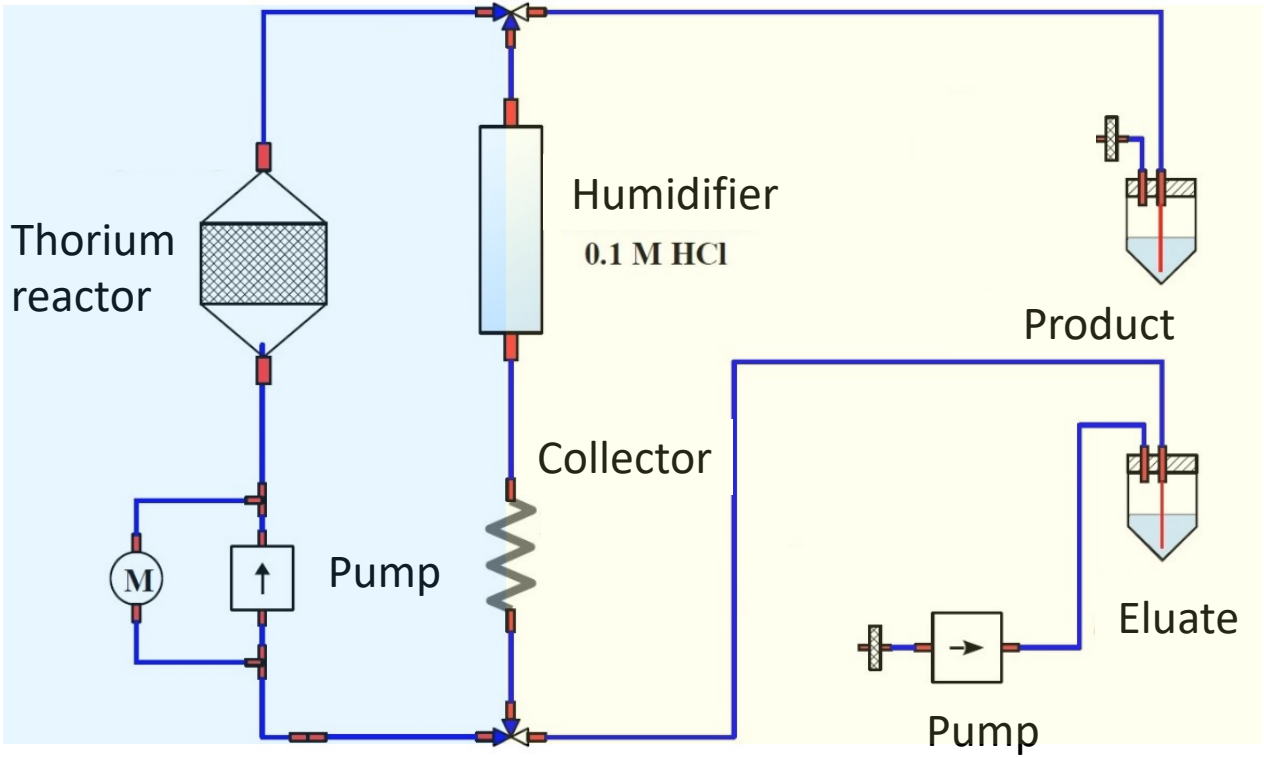
Advantages of α-emitters:

- The passage of 2-4 α-particles through the nucleus causes death with a probability of 40%
- Efficient in small clusters, reducing the likelihood of recurrence

Benefits of ^{212}Pb:

- ^{212}Pb β-emitter ($T_{1/2}$ 10.64 h) – *in vivo* ^{212}Bi α-emitter generator ($T_{1/2}$ 1 h)
- The presence of a diagnostic pair ^{203}Pb (γ-line 279 keV)

$^{228}Th/^{212}Pb$ generator

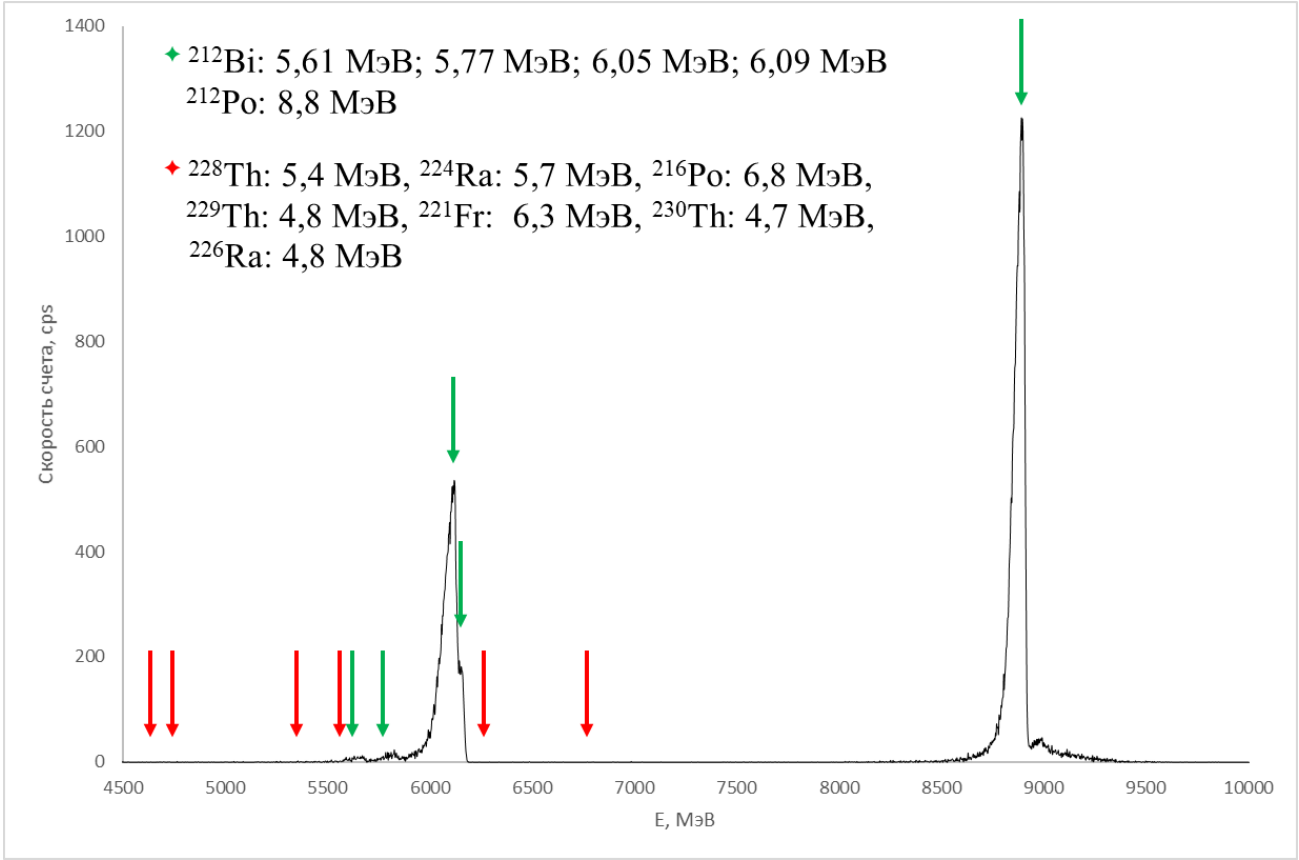


$^{228}Th/^{212}Pb$ generator

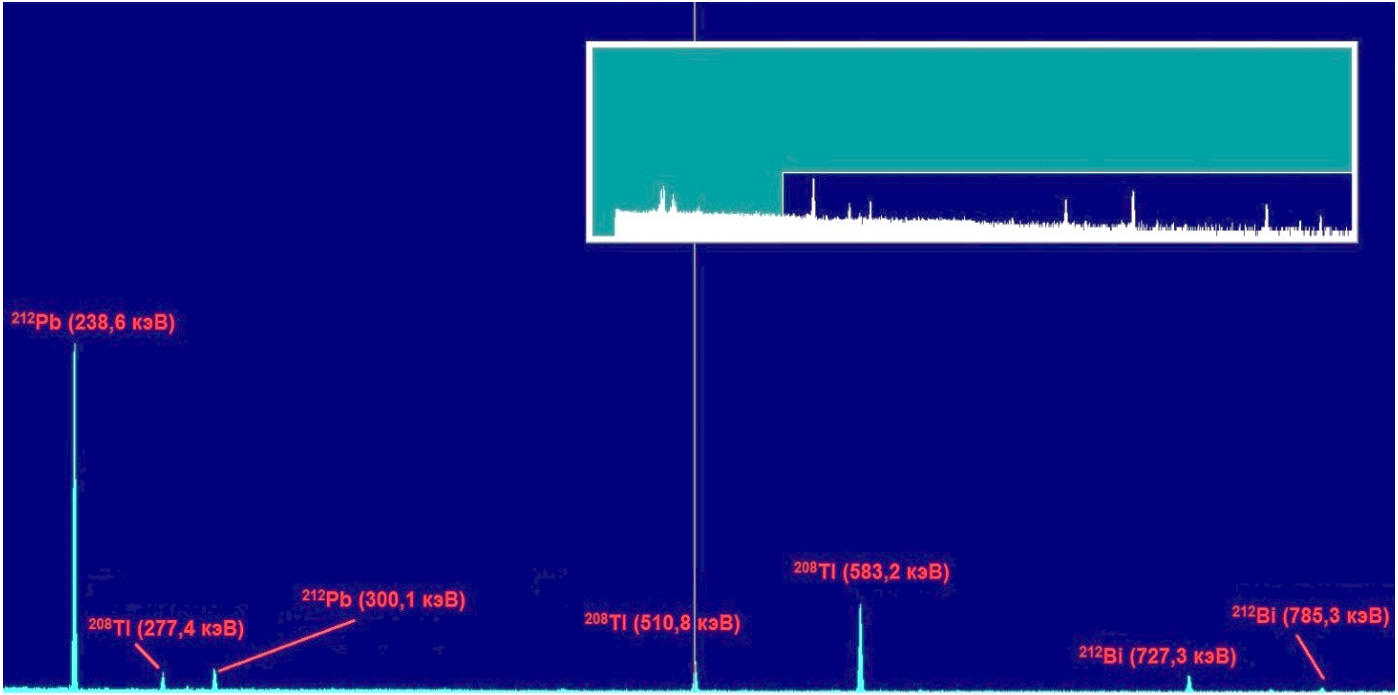
Generator Product

Parameter	Value		
pH	1,0		
Volume activity	max 4.0 MBq/ml		
Radioactive impurities	Less than detection limit		
Chemical impurities	**Fe**	0.12	ppm
	Pb	0.05	ppm
	Cu	0.005	ppm
	Zn	0.003	ppm
	As	0.13	ppm
	Others	<0.5	ppm
Eluate	0.1 M HCl		
Half-life	10.64 h		
Description	Transparent liquid		

Alpha-Spectrum of Generator Product



Gamma-Spectrum of Generator Product



High-purity germanium detector (ORTEC)

Targeted Alpha Therapy: DOTATATE

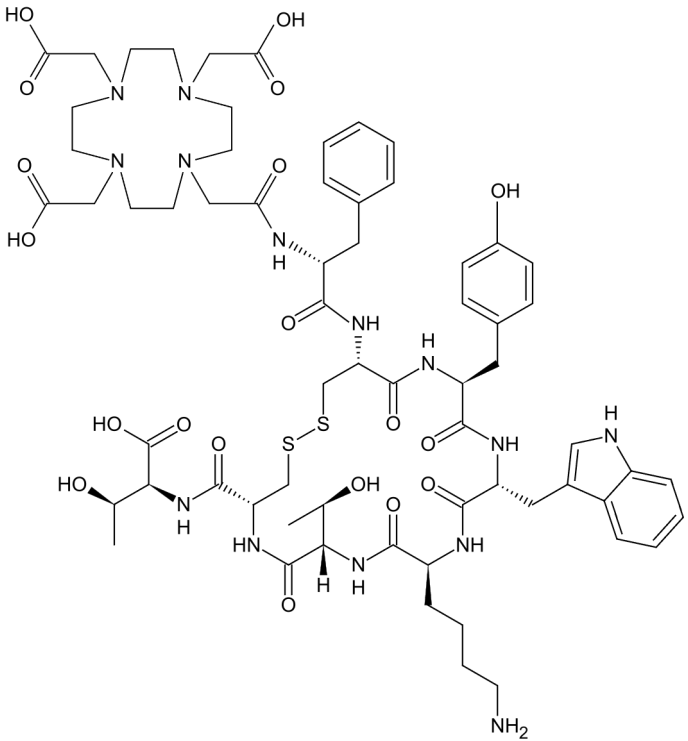
DOTATATE
(DOTA-(Tyr^3)-octreotate)

Synthetic octapeptide DOTATATE (DOTA-DPhe-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr)

1,0 mg/ml

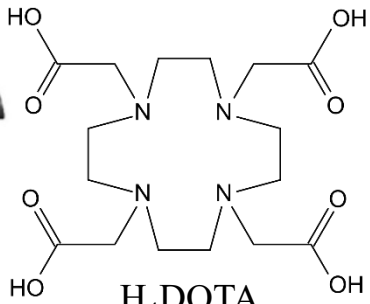
1,5 kDa

High affinity to **SSTR-antigen** (neuroendocrine tumors)

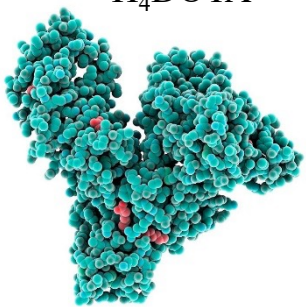


Targeted Alpha Therapy: DARPin9_29

DARPin9_29



H_4DOTA



ЧСА

DARPin, англ. «designed ankyrin repeat proteins»

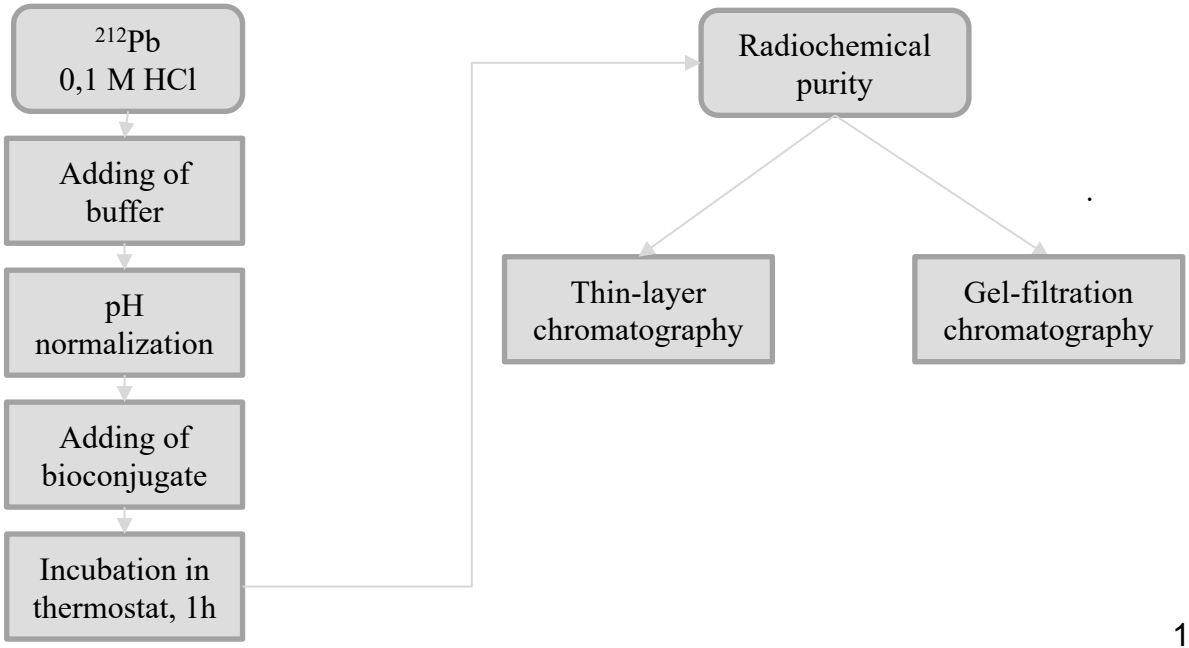
DOTA-HSA-DARPin9_29
(S.M. Deev, RAS)

Conjugate of DARPin (8 kDa), human serum albumine (HSA) and chelator DOTA

1,0 mg/ml
95 kDa
High affinity to **HER2-antigen** (breast cancer)

Complexation with HSA (69 kDa) increases half-life in blood: if nanoparticle size is less than 60-65 kDa, the particle lifetime in blood is only 1,5-2 h!

Radiolabeling and Preparation

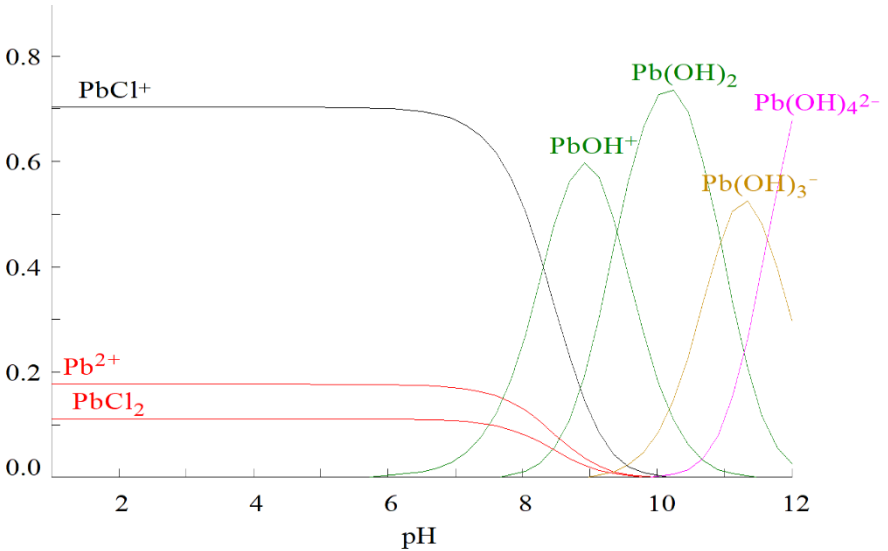


Synthesis [^{212}Pb]DOTATATE

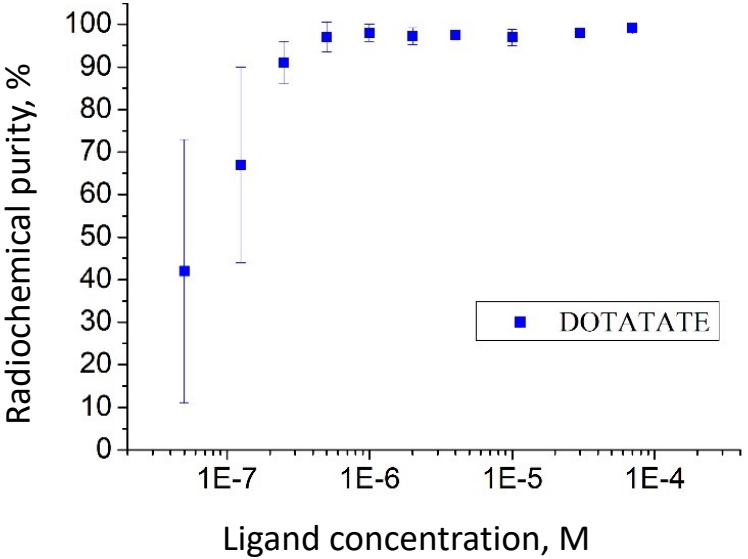
[^{212}Pb]DOTATATE:

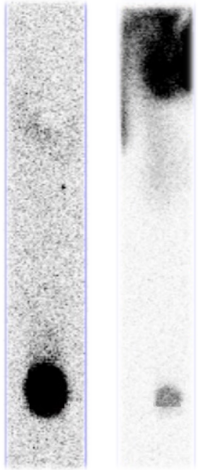
- Complexation within pH > 5.0
- ^{212}Pb in 0,1 M HCl (pH 1,0)
- 2 M Na_2CO_3 for pH 5,5
- DOTATATE $\leq 7 \cdot 10^{-5}$ M

- Raising the pH to deprotonate and prevent DOTATATE from entering a strongly acidic environment
- Adding buffer to prevent hydrolysis, maintain ionic form while increasing pH



Synthesis of [^{212}Pb]DOTATATE





[^{210}Pb]DOTATATE (left)

[^{210}Pb]DTPA (right)

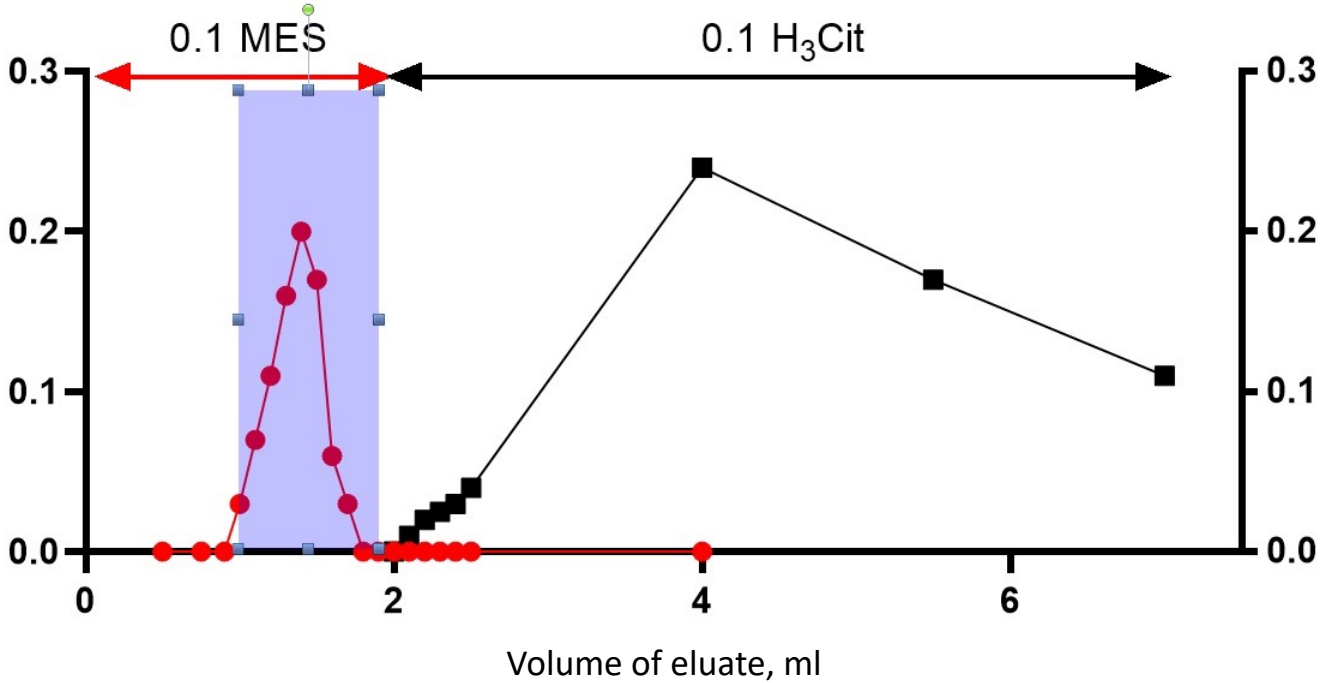
^{212}Pb in 0.1 M HCl, pH 1.0

10^{-10} M of ^{212}Pb

$5 \cdot 10^{-8}$ M ≤ DOTATATE ≤ 10^{-4} M

T = 90^{0}C, t = 60 min

Synthesis of [^{212}Pb]DOTA-HSA-DARPin9_29



- Peak separation at 2 ml eluate volume (fractionation threshold 5 kDa)
- The radiochemical purity at the time of fraction separation was 82 ± 5%

Stability of Complexes

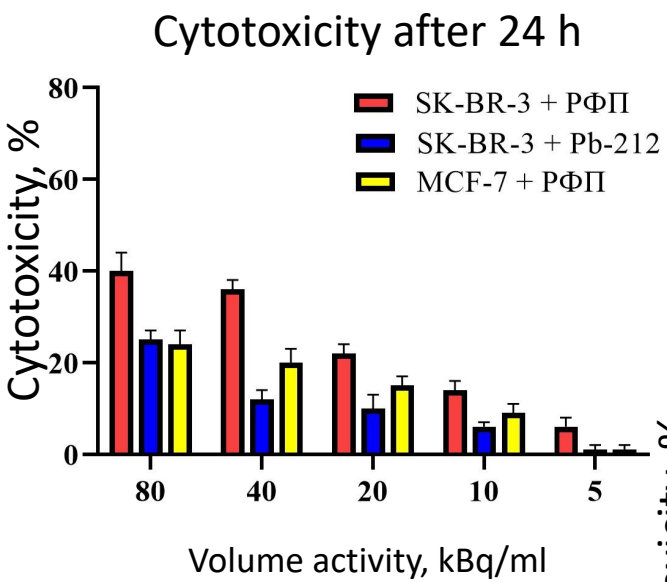
[^{212}Pb]DOTATATE

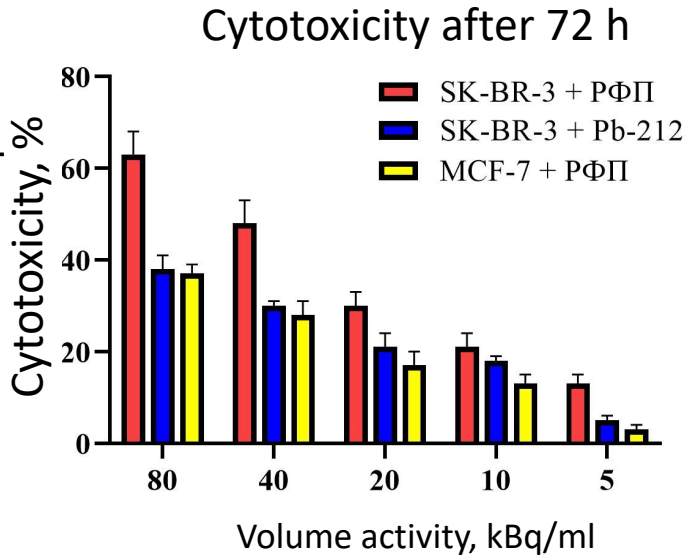
Medium	1 h	3 h	6 h	10 h
Serum (1:10)	83 ± 5	77 ± 7	84 ± 7	88 ± 5
NaCl 0,9%	96 ± 3	95 ± 5	94 ± 4	96 ± 4

[^{212}Pb]DOTA-HSA-DARPin9_29

Medium	1 h	2 h	3 h
Serum (1:1)	96 ± 3	99 ± 5	94 ± 3
NaCl 0,9%	92 ± 3	93 ± 5	94 ± 4

Cytotoxic Effect *in vitro*





Summary

1. A laboratory generator has been developed that makes it possible to continuously obtain ^{212}Pb with a yield of up to 50%. The content of long-lived parental radionuclides in the ^{212}Pb solution is a value that does not exceed the detection threshold on the α-spectrometer, which makes it possible to exclude operations for its purification.

2. It was shown that the complexation of ^{212}Pb and the DOTATATE and DOTA-HSA-DARPin9_29 molecules occurs efficiently (radiochemical purity max 99% and 85%, respectively) at synthesis temperatures of 90^0C and 60^0C, respectively.

3. Stability of the synthesized complexes remains at a level of at least 95% in isotonic solution, and in blood serum - at least 80-85%, which indicates prolonged therapeutic potential of the synthesized compounds over time.

4. The cytotoxic effect of the synthesized compound based on ^{212}Pb and DARPin9_29 on target cancer cells was also demonstrated.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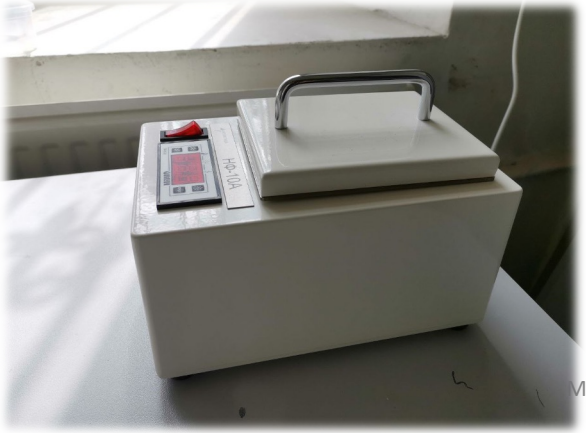
HP-10A


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Thank you for attention!