ZSJPL005



Compound	Amount	Final conc.		
Fluorinase enzyme	5 mg in 110 $\mu$ L water (PO <sub>4</sub> <sup>3-</sup>			
	buffer, 50 mM), 174 nmol			
L-SeMet (2mM in water)	40 μL, 80 nmol			
Biotin-CIDA	0.4 mg in 50 μL, 540 nmol			
<sup>18</sup> F in O18 Water	50 μL	457 MBq @ 13:01		

12 mins bombardment on T4, no rinse, final activity 186 approx. 8.1 GBq

- 13:01 start incubation 37 °C (457 MBq)
- reaction mixture was boiled at 95°C for 5 min (371 Mbq @ 13:31). After that 250 μL of water ere added and the eppendorf was spinned at 13500 rpm for 5 min. 345 MBq @ 13:44.
  Approx. 450 μL of the supernatant (289 MBq @ 13:46 were injected in the HPLC (8.7 MBq residual in the syringe @ 13:48). Approx. 48.8 MBq @ 13:48 left in the Eppendorf.
- Collected 73.4 MBq @ 14:00, approx. 26% of injected activity (Rt 9.66 min)

## semiprep crude radio signal



Peak#	Ret. Time	Area	Height	Mark	Conc.	Area%
1	3.796	1170833	38346	Μ	64.165	64.165
2	6.816	3292	830	Μ	0.180	0.180
3	9.655	634761	116529	М	34.787	34.787
4	9.858	15823	2253	VΜ	0.867	0.867
Total		1824710	157958		100.000	100.000

Solution in the collection vial (73.4 MBq @ 14:00) was diluted with 50 mL of water and content loaded on the OASIS HLB light cartridge

66.6 MBq trapped in the cartridge @ 14:07

- 1.2 MBq residue in the collection vial @ 14:08
- 1.8 MBq in the filtered solution @ 14:09

Cartridge was washed with 20 mL of water

65.0 Mbq trapped in the cartridge @14:12

1.1 MBq in the wash solution @14:13

Activity was eluted from the cartridge with 0.4 mL of Ethanol 52.3 MBq eluted @14:18 10.4 MBq left in the cartridge @14:16

Activity was eluted again from the cartridge with 0.2 mL of Ethanol 7.4 MBq eluted @14:19 3.5 MBq left in the cartridge @14:17

100  $\mu$ L of Ethanol solution were diluted with water to 500  $\mu$ L, 100  $\mu$ L of this solution,1.66 MBq @ 14:23 were injected in HPLC analytical, 0.05 MBq @ 14:26 left in the syringe. Analytical peak collected 1.35 MBq @14:31

Radio HPLC Diluted 1:5 solution



100  $\mu L$  of 1:5 diluted solution were diluted with water to 500  $\mu L$ , 100  $\mu L$  of this solution was injected again in the HPLC.



### Radio HPLC of 1:25 solution

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%
1	4.374	293064	51119	М	10.948				10.948
2	5.723	2383893	408725	М	89.052				89.052
Total		2676957	459844		100.000				100.000

Radiochemical purity is approx. 90%

### **Avidin binding TEST**

Vial A 0.56mg Avidin diluted in 300  $\mu$ L PBS buffer + 50  $\mu$ L of solution of ZSJPL005 pure diluted 1:5 with water (0.8 MBq @15:03) Vial B 300  $\mu$ L PBS buffer + 50  $\mu$ L of solution of ZSJPL005 pure diluted 1:5 with water (0.8 MBq @15:04)

Both incubated for 30 min at 37 °C. Vial A 0.55 MBq @15:37 Vial B 0.53 MBq @15:38

Then 50  $\mu\text{L}$  of solution were spotted on a TLC plate and TLC eluted with solution 75% ACN/25% MeOH/5% Water.

#### TLC of solution A



96% of activity in region1 with Rf 0

# TLC of solution B

Measurement ZS. C:\GINA_NT\FDG(	JPLOOS TI 2C\ZSJPL(	SST-B S 005 TES	TREPT/ T-8 S1	WIDINBIS	LONG2.	rta XNG2.rt	rayte a Pr	st int date	Page 1/1 13/03/2017
46 C/mm g 44 - Di 42 - So 40 - 38 - 36 - 34 -				Reg #2					R tone
30- 28 26- 24- 22									
20-				Section 1	111				
18-	1		1		10 M				
16	2.0	121 12	N		植圆	1			
34		1 1	1000						
12	- ME #	1.1							
10	- 1844								
8 8	100.00	10 ani			100				
6	A TRACE	S. Ne				B			
	1000					alal			
	122.00						nn	1.1.1	AAA
0	20		0	60	1. 1.	80		00	120 mm
Sample descript	ion	28	8						110 111
Measurement:	2.SJI	2L005 T	EST-B	STREPTAVIO	INBIS	BLONG2.	rta, star	teda	
Nethod: FDGQC	723								
Vergine 10 mm	\$100	IT 125 1	NUM			1222	-		
High voltage:	550.	0 V	Negola	ition:	0.	/ mm	Energy	1.2	
Bad return valu	e of com	nand MT							
Radio detector:	raytest	MiniGI	TA	Serial	Nr.: 3	2006156			
Integration TLC									
Substance	R/F	%Total	Type	Area	tArea				
and the second second	28 (160)			Counts	8				
Reg #1	-0,012	5,08	DD	58.000	5.32				
Reg #2	0.387	90.53	80	1033.000	94,68				
Sum in POT				1091 000					
Total area				1141.000					
Area RF				1129.000					
rea Mr.				1123.000					

90% of activity in reagion 2, only 5% under the area where the activity is located when streptavidin is present.