

SEQUENCE LISTING

<110> National University of Ireland, Galway

<120> The detection of free and protein-bound non-human Gal-Î±-(1â†'3)-Gal epitope

<130> PT331PCT

<160> 11

<170> BiSSAP 1.2

<210> 1

<211> 266

<212> PRT

<213> Artificial Sequence

<220>

<223> Generic sequence for engineered single chain antibody fragment for detecting non-human Gal-Î±-(1â†'3)-Gal carbohydrate epitope

<220>

<221> VARIANT

<222> 12

<223> X is T or A

<220>

<221> VARIANT

<222> 16

<223> X is G or E

<220>

<221> VARIANT

<222> 26..33

<223> X is a multi aa sequence comprising [N or G or absent], [S or absent], [Y or absent], [G or absent], [G or absent], [S or absent], [G or Y] and [N or H or Y]

<220>

<221> VARIANT

<222> 54.55

<223> X is a multi aa sequence comprising [N or D] and [K or Q or N]

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<221> VARIANT

<222> 59

<223> X is D or G

<220>

<221> VARIANT

<222> 68

<223> X is T or K

<220>

<221> VARIANT

<222> 70

<223> X is G or D

<220>

<221> VARIANT

<222> 73
<223> X is A or G or S

<220>
<221> VARIANT
<222> 82
<223> X is A or V

<220>
<221> VARIANT
<222> 89
<223> X is F or Y

<220>
<221> VARIANT
<222> 92
<223> X is A or S or T

<220>
<221> VARIANT
<222> 95..98
<223> X is a multi aa sequence comprising [N or S], [S or absent], [N or absent] and [T or S]

<220>
<221> VARIANT
<222> 100
<223> X is V or A

<220>
<221> VARIANT
<222> 102
<223> X is V or I

<220>
<221> VARIANT
<222> 108
<223> X is T or A

<220>
<221> VARIANT
<222> 120
<223> X is S or absent

<220>
<221> VARIANT
<222> 141
<223> X is L or F

<220>
<221> VARIANT
<222> 147
<223> X is G or A

<220>
<221> VARIANT
<222> 170
<223> X is T or A

<220>
<221> VARIANT

<222> 183
<223> X is Y or N

<220>
<221> VARIANT
<222> 186
<223> X is S or R

<220>
<221> VARIANT
<222> 207
<223> X is N or S

<220>
<221> VARIANT
<222> 209
<223> X is V or L

<220>
<221> VARIANT
<222> 225
<223> X is Y or F

<220>
<221> VARIANT
<222> 230
<223> X is A or G

<220>
<221> VARIANT
<222> 232..234
<223> X is a multi aa sequence comprising [T or S], [I or G] and [Y or N]

<220>
<221> VARIANT
<222> 247
<223> X is D or N

<400> 1
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1 5 10 15
Thr Val Lys Ile Thr Cys Ser Gly Gly Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30
Xaa Tyr Gly Trp Tyr Gln Gln Lys Ser Pro Gly Ser Ala Pro Val Thr
35 40 45
Val Ile Tyr Ser Asn Xaa Xaa Arg Pro Ser Xaa Ile Pro Ser Arg Phe
50 55 60
Ser Gly Ser Xaa Ser Xaa Ser Thr Xaa Thr Leu Thr Ile Thr Gly Val
65 70 75 80
Gln Xaa Asp Asp Glu Ala Val Tyr Xaa Cys Gly Xaa Tyr Asp Xaa Xaa
85 90 95
Xaa Xaa Tyr Xaa Gly Xaa Phe Gly Ala Gly Thr Xaa Leu Thr Val Leu
100 105 110
Gly Gln Ser Ser Arg Ser Ser Xaa Gly Gly Gly Ser Ser Gly Gly Gly
115 120 125
Gly Ser Ala Val Thr Leu Asp Glu Ser Gly Gly Gly Xaa Gln Thr Pro
130 135 140
Gly Gly Xaa Leu Ser Leu Val Cys Lys Ala Ser Gly Phe Thr Phe Ser
145 150 155 160
Ser Tyr Ser Met Gln Trp Val Arg Gln Xaa Pro Gly Lys Gly Leu Glu

				165				170					175			
Phe	Val	Ala	Gly	Ile	Gly	Xaa	Ser	Asp	Xaa	Tyr	Thr	Tyr	Phe	Gly	Pro	
			180					185					190			
Ala	Val	Lys	Gly	Arg	Ala	Thr	Ile	Ser	Arg	Asp	Asn	Gly	Gln	Xaa	Thr	
		195					200					205				
Xaa	Arg	Leu	Gln	Leu	Asn	Asn	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Thr	Tyr	
	210				215						220					
Xaa	Cys	Ala	Arg	Ser	Xaa	Asp	Xaa	Xaa	Xaa	Gly	Cys	Thr	His	Pro	Trp	
225					230					235					240	
Cys	Ser	Ala	Asp	Asn	Ile	Xaa	Ala	Trp	Gly	His	Gly	Thr	Glu	Val	Ile	
			245					250						255		
Val	Ser	Ser	Thr	Ser	Gly	Gln	Ala	Gly	Gln							
			260					265								

<210> 2
 <211> 259
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> scFv-A4

<400> 2

Gln	Ala	Ala	Leu	Thr	Gln	Pro	Ser	Ser	Val	Ser	Thr	Asn	Pro	Gly	Gly	
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Thr	Val	Lys	Ile	Thr	Cys	Ser	Gly	Gly	Asn	Gly	Asn	Tyr	Gly	Trp	Tyr	
		20					25					30				
Gln	Gln	Lys	Ser	Pro	Gly	Ser	Ala	Pro	Val	Thr	Val	Ile	Tyr	Ser	Asn	
		35				40					45					
Asn	Lys	Arg	Pro	Ser	Asp	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Lys	Ser	
	50				55				60							
Gly	Ser	Thr	Ala	Thr	Leu	Thr	Ile	Thr	Gly	Val	Gln	Val	Asp	Asp	Glu	
65					70				75						80	
Ala	Val	Tyr	Phe	Cys	Gly	Ala	Tyr	Asp	Asn	Thr	Tyr	Val	Gly	Val	Phe	
			85					90					95			
Gly	Ala	Gly	Thr	Thr	Leu	Thr	Val	Leu	Gly	Gln	Ser	Ser	Arg	Ser	Ser	
			100				105						110			
Ser	Gly	Gly	Gly	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Val	Thr	Leu	Asp	
	115					120					125					
Glu	Ser	Gly	Gly	Gly	Leu	Gln	Thr	Pro	Gly	Gly	Gly	Leu	Ser	Leu	Val	
	130				135						140					
Cys	Lys	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ser	Met	Gln	Trp	Val	
145					150					155					160	
Arg	Gln	Thr	Pro	Gly	Lys	Gly	Leu	Glu	Phe	Val	Ala	Gly	Ile	Gly	Tyr	
			165					170					175			
Ser	Asp	Ser	Tyr	Thr	Tyr	Phe	Gly	Pro	Ala	Val	Lys	Gly	Arg	Ala	Thr	
	180						185						190			
Ile	Ser	Arg	Asp	Asn	Gly	Gln	Asn	Thr	Val	Arg	Leu	Gln	Leu	Asn	Asn	
	195					200						205				
Leu	Arg	Ala	Glu	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys	Ala	Arg	Ser	Ala	Asp	
	210				215						220					
Thr	Ile	Tyr	Gly	Cys	Thr	His	Pro	Trp	Cys	Ser	Ala	Asp	Asn	Ile	Asp	
225					230				235						240	
Ala	Trp	Gly	His	Gly	Thr	Glu	Val	Ile	Val	Ser	Ser	Thr	Ser	Gly	Gln	
				245				250						255		

Ala Gly Gln

<210> 3
 <211> 261
 <212> PRT

<213> Artificial Sequence

<220>

<223> scFv-G12

<400> 3

Gln	Ala	Ala	Leu	Thr	Gln	Pro	Ser	Ser	Val	Ser	Ala	Asn	Pro	Gly	Glu
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Thr	Val	Lys	Ile	Thr	Cys	Ser	Gly	Gly	Ser	Tyr	His	Tyr	Gly	Trp	Tyr
			20					25					30		
Gln	Gln	Lys	Ser	Pro	Gly	Ser	Ala	Pro	Val	Thr	Val	Ile	Tyr	Ser	Asn
		35					40					45			
Asn	Gln	Arg	Pro	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Thr	Ser
	50					55				60					
Asp	Ser	Thr	Gly	Thr	Leu	Thr	Ile	Thr	Gly	Val	Gln	Ala	Asp	Asp	Glu
65					70					75					80
Ala	Val	Tyr	Phe	Cys	Gly	Ser	Tyr	Asp	Ser	Ser	Asn	Thr	Tyr	Ala	Gly
				85					90					95	
Ile	Phe	Gly	Ala	Gly	Thr	Thr	Leu	Thr	Val	Leu	Gly	Gln	Ser	Ser	Arg
			100					105					110		
Ser	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Val	Thr
		115					120					125			
Leu	Asp	Glu	Ser	Gly	Gly	Gly	Phe	Gln	Thr	Pro	Gly	Gly	Ala	Leu	Ser
	130					135					140				
Leu	Val	Cys	Lys	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ser	Met	Gln
145					150					155					160
Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Phe	Val	Ala	Gly	Ile
			165						170					175	
Gly	Asn	Ser	Asp	Arg	Tyr	Thr	Tyr	Phe	Gly	Pro	Ala	Val	Lys	Gly	Arg
			180					185					190		
Ala	Thr	Ile	Ser	Arg	Asp	Asn	Gly	Gln	Ser	Thr	Leu	Arg	Leu	Gln	Leu
		195					200					205			
Asn	Asn	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys	Ala	Arg	Ser
	210					215					220				
Gly	Asp	Ser	Gly	Asn	Gly	Cys	Thr	His	Pro	Trp	Cys	Ser	Ala	Asp	Asn
225					230					235					240
Ile	Asn	Ala	Trp	Gly	His	Gly	Thr	Glu	Val	Ile	Val	Ser	Ser	Thr	Ser
			245						250					255	
Gly	Gln	Ala	Gly	Gln											
			260												

<210> 4

<211> 263

<212> PRT

<213> Artificial Sequence

<220>

<223> scFv-A11

<400> 4

Gln	Ala	Ala	Leu	Thr	Gln	Pro	Ser	Ser	Val	Ser	Ala	Asn	Pro	Gly	Glu
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Thr	Val	Lys	Ile	Thr	Cys	Ser	Gly	Gly	Gly	Ser	Tyr	Gly	Gly	Ser	Tyr
			20					25					30		
Tyr	Tyr	Gly	Trp	Tyr	Gln	Gln	Lys	Ser	Pro	Gly	Ser	Ala	Pro	Val	Thr
		35					40					45			
Val	Ile	Tyr	Ser	Asn	Asp	Asn	Arg	Pro	Ser	Asp	Ile	Pro	Ser	Arg	Phe
	50					55				60					
Ser	Gly	Ser	Thr	Ser	Gly	Ser	Thr	Ser	Thr	Leu	Thr	Ile	Thr	Gly	Val
65					70					75					80
Gln	Val	Asp	Asp	Glu	Ala	Val	Tyr	Tyr	Cys	Gly	Thr	Tyr	Asp	Ser	Ser

				85					90					95					
Tyr	Val	Gly	Ile	Phe	Gly	Ala	Gly	Thr	Ala	Leu	Thr	Val	Leu	Gly	Gln				
			100					105					110						
Ser	Ser	Arg	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Ala				
		115					120						125						
Val	Thr	Leu	Asp	Glu	Ser	Gly	Gly	Gly	Leu	Gln	Thr	Pro	Gly	Gly	Gly				
	130					135					140								
Leu	Ser	Leu	Val	Cys	Lys	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ser				
145					150					155					160				
Met	Gln	Trp	Val	Arg	Gln	Thr	Pro	Gly	Lys	Gly	Leu	Glu	Phe	Val	Ala				
				165					170						175				
Gly	Ile	Gly	Tyr	Ser	Asp	Ser	Tyr	Thr	Tyr	Phe	Gly	Pro	Ala	Val	Lys				
			180				185						190						
Gly	Arg	Ala	Thr	Ile	Ser	Arg	Asp	Asn	Gly	Gln	Asn	Thr	Val	Arg	Leu				
		195					200						205						
Gln	Leu	Asn	Asn	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys	Ala				
	210					215						220							
Arg	Ser	Ala	Asp	Thr	Ile	Tyr	Gly	Cys	Thr	His	Pro	Trp	Cys	Ser	Ala				
225					230					235					240				
Asp	Asn	Ile	Asp	Ala	Trp	Gly	His	Gly	Thr	Glu	Val	Ile	Val	Ser	Ser				
				245					250						255				
Thr	Ser	Gly	Gln	Ala	Gly	Gln													
			260																

<210> 5

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Variable Light Chain Sequence 1

<220>

<221> VARIANT

<222> 4

<223> X is N or G or absent

<220>

<221> VARIANT

<222> 5

<223> X is S or absent

<220>

<221> VARIANT

<222> 6

<223> X is Y or absent

<220>

<221> VARIANT

<222> 7

<223> X is GG or absent

<220>

<221> VARIANT

<222> 8

<223> X is S or absent

<220>

<221> VARIANT

<222> 9

<223> X is Y or G or absent

<220>
<221> VARIANT
<222> 10
<223> X is N or Y

<400> 5
Ser Gly Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr
1 5 10

<210> 6
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable Light Chain Sequence 2

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<221> VARIANT
<222> 3
<223> X is N or D

<220>
<221> VARIANT
<222> 4
<223> X is K or N or Q

<400> 6
Ser Asn Xaa Xaa Arg Pro Ser
1 5

<210> 7
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable Light Chain Sequence 3

<220>
<221> VARIANT
<222> 2
<223> X is A, T or S

<220>
<221> VARIANT
<222> 5
<223> X is S or N

<220>
<221> VARIANT
<222> 6
<223> X is S or absent

<220>
<221> VARIANT
<222> 7
<223> X is N or absent

<220>

<221> VARIANT
<222> 8
<223> X is T or S

<220>
<221> VARIANT
<222> 10
<223> X is V or A

<400> 7
Gly Xaa Tyr Asp Xaa Xaa Xaa Xaa Tyr Xaa Gly Val
1 5 10

<210> 8
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable heavy chain sequence 5

<400> 8
Gly Phe Thr Phe Ser Ser Tyr Ser Met Gln
1 5 10

<210> 9
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
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<223> X is Y or N

<220>
<223> Variable heavy chain sequence 6

<220>
<221> VARIANT
<222> 4
<223> X is S or R

<400> 9
Xaa Ser Asp Xaa Tyr Thr Tyr Phe Gly Pro Ala Val Lys Gly
1 5 10

<210> 10
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable heavy chain sequence 7

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<221> VARIANT
<222> 2
<223> X is A or G

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<221> VARIANT
<222> 4
<223> X is TIY or SGN

<220>
<221> VARIANT
<222> 17
<223> X is D or N

<400> 10
Ser Xaa Asp Xaa Gly Cys Thr His Pro Trp Cys Ser Ala Asp Asn Ile
1 5 10 15
Xaa Ala

<210> 11
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Sequence 9

<220>
<221> VARIANT
<222> 8
<223> X is S or absent

<400> 11
Gly Gln Ser Ser Arg Ser Ser Xaa Gly Gly Gly Ser Ser Gly Gly Gly
1 5 10 15
Gly Ser