In-line Uranium Immunosensor

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Immunosensors: Effective Analytical Tools for Environmental Analysis

- Provide rapid, near real-time results at the site of contamination
- Used to map contaminated sites and to quickly monitor the effectiveness of bioremediation and containment efforts
- Used in the analytical laboratory to rank samples by concentration before additional instrumental analysis

Antibody-Based Assays Are Cost-Effective

- Sample analysis is one of the major costs associated with the remediation of a contaminated site
- Studies have shown that the use of antibody-based assays can reduce analysis costs by 50% or more

Antibody-Based Assays Are Simple to Perform

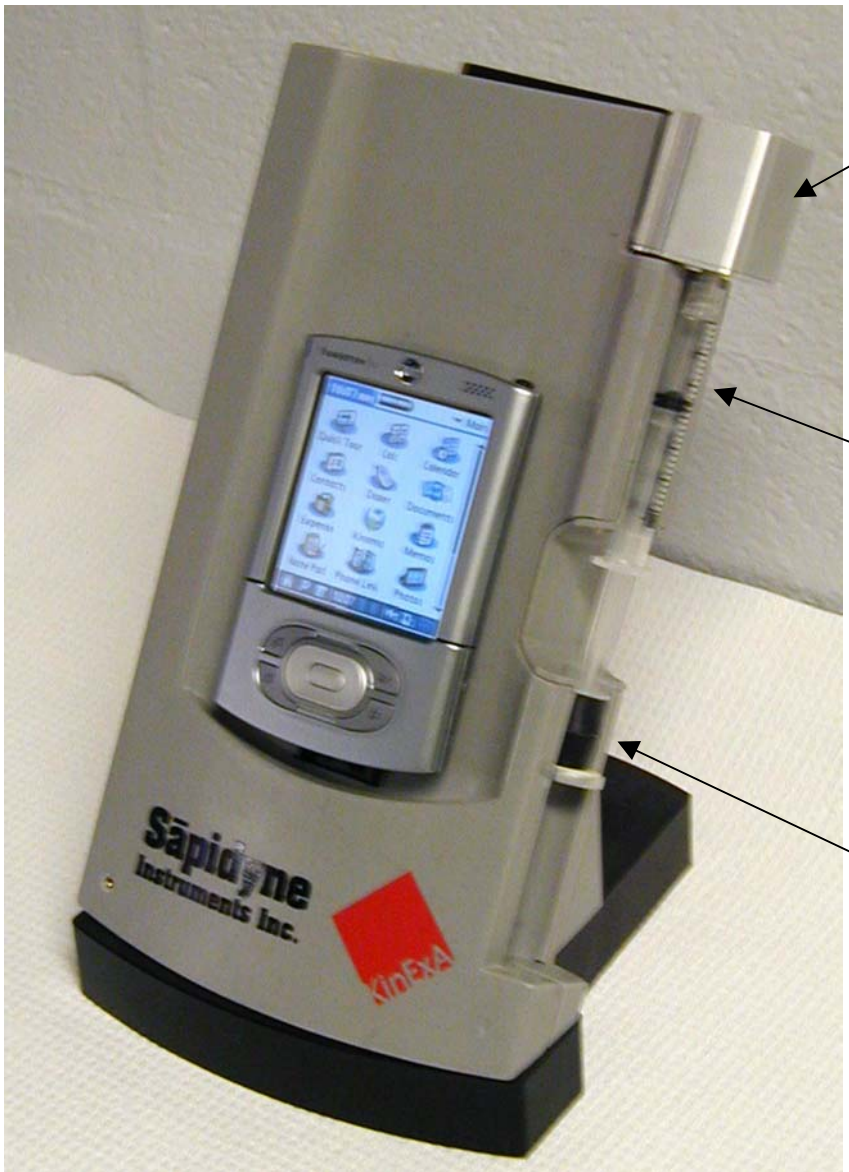
- No complicated, sophisticated instruments are required
- Individuals performing the assays do not need to be highly skilled in analytical techniques
- Sample preparation procedures are usually uncomplicated and require minimal solvent

Project Goals

- To develop an in-line uranium immunosensor that can be used to determine the efficacy of specific biostimulation processes

The sensor will be designed to autonomously:

- Self-calibrate using standard reagents;
- Collect a sample from a process line;
- Measure U(VI) at varying times during the treatment process.

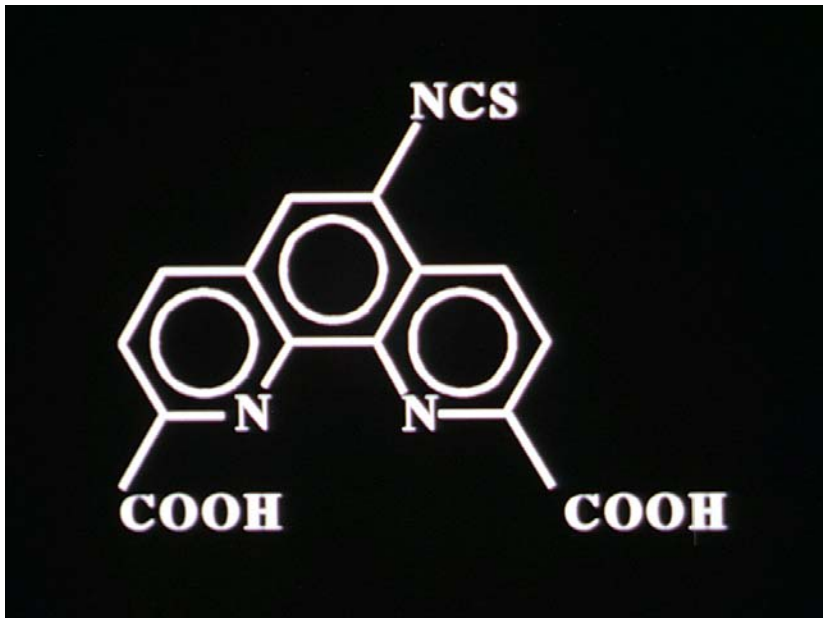


Optics, LED, and capillary bed containing particles with immobilized capture ligand.

Fluorescently labeled antibody and environmental contaminant are mixed in a disposable syringe.

Drive motor pushes the antibody-contaminant mixture over the capture ligand.

Generation of UO_2^{2+}-specific monoclonal antibodies using a 2,9-dicarboxyl-1,10-phenanthroline (DCP) derivative

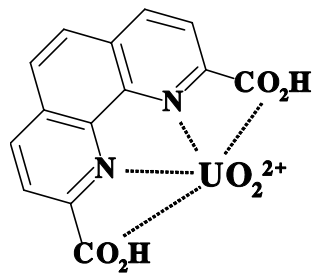


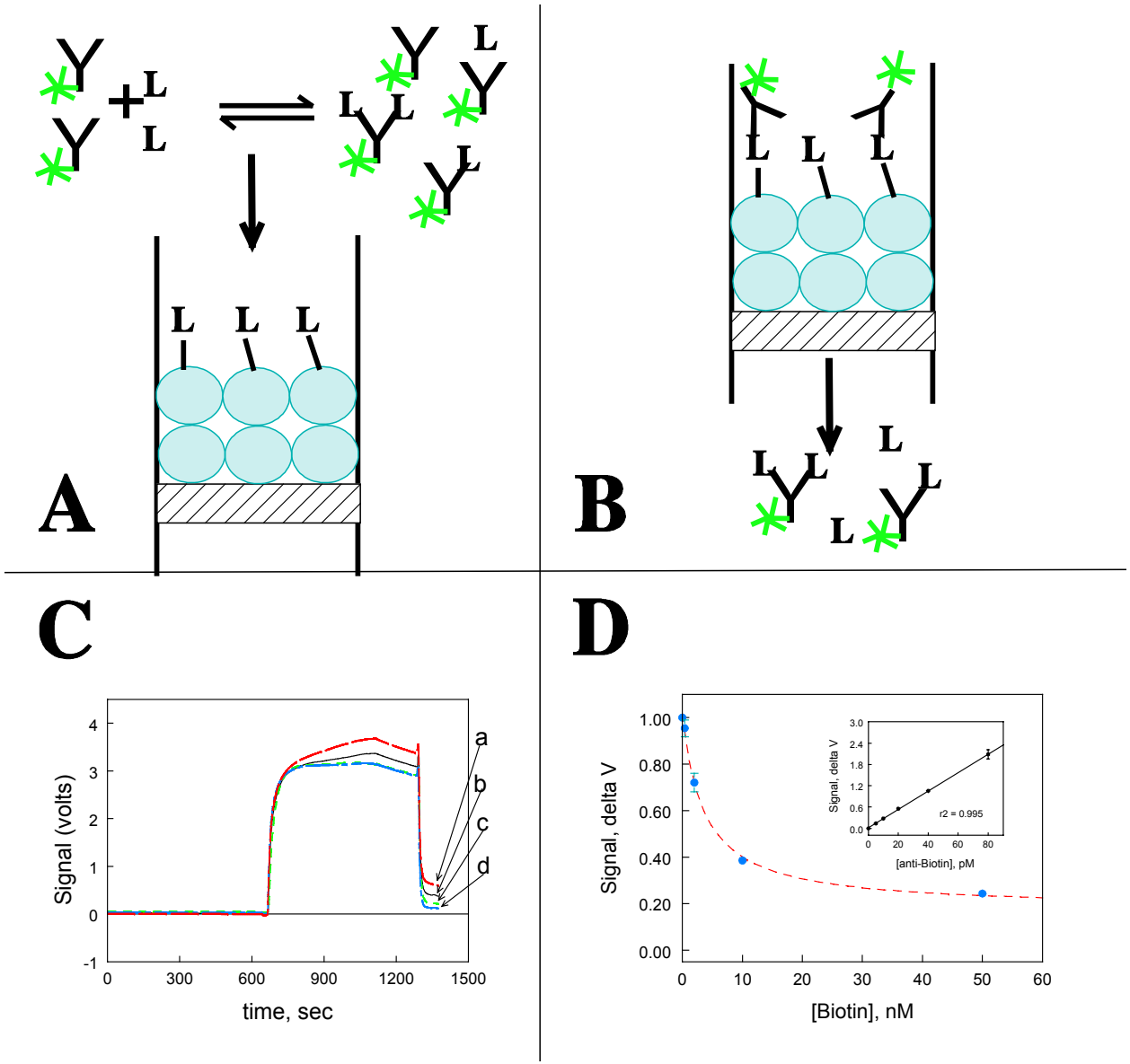
1. Prepare DCP-protein conjugate
2. Load with UO_2^{2+}
3. Inject mice and wait for immune response to develop
4. Harvest spleen cells and make hybridomas
5. Screen hybridomas for antibodies with desirable characteristics for incorporation into sensors

Desirable Characteristics for Metal-Specific Antibodies

- Little or no binding to the metal-free chelator
- Tight binding to the chelated metal of interest
- Little or no binding to other metals most likely to contaminate the environmental sample
- Little or no interference from components likely to be present in the environmental sample matrix

Equilibrium dissociation constants for the binding of 2,9-dicarboxyl-1,10-phenanthroline (DCP) in the presence and absence of uranyl ion to monoclonal antibodies 12F6, 10A3, and 8A11.

	Value of K_d, M		
Ligand	**12F6**	**10A3**	**8A11**
	$9.1 \pm 0.7 \times 10^{-10}$	$2.4 \pm 0.2 \times 10^{-9}$	$5.5 \pm 0.2 \times 10^{-9}$
Metal-free DCP	$7.5 \pm 0.5 \times 10^{-7}$	$2.8 \pm 0.1 \times 10^{-6}$	$3.7 \pm 0.2 \times 10^{-6}$

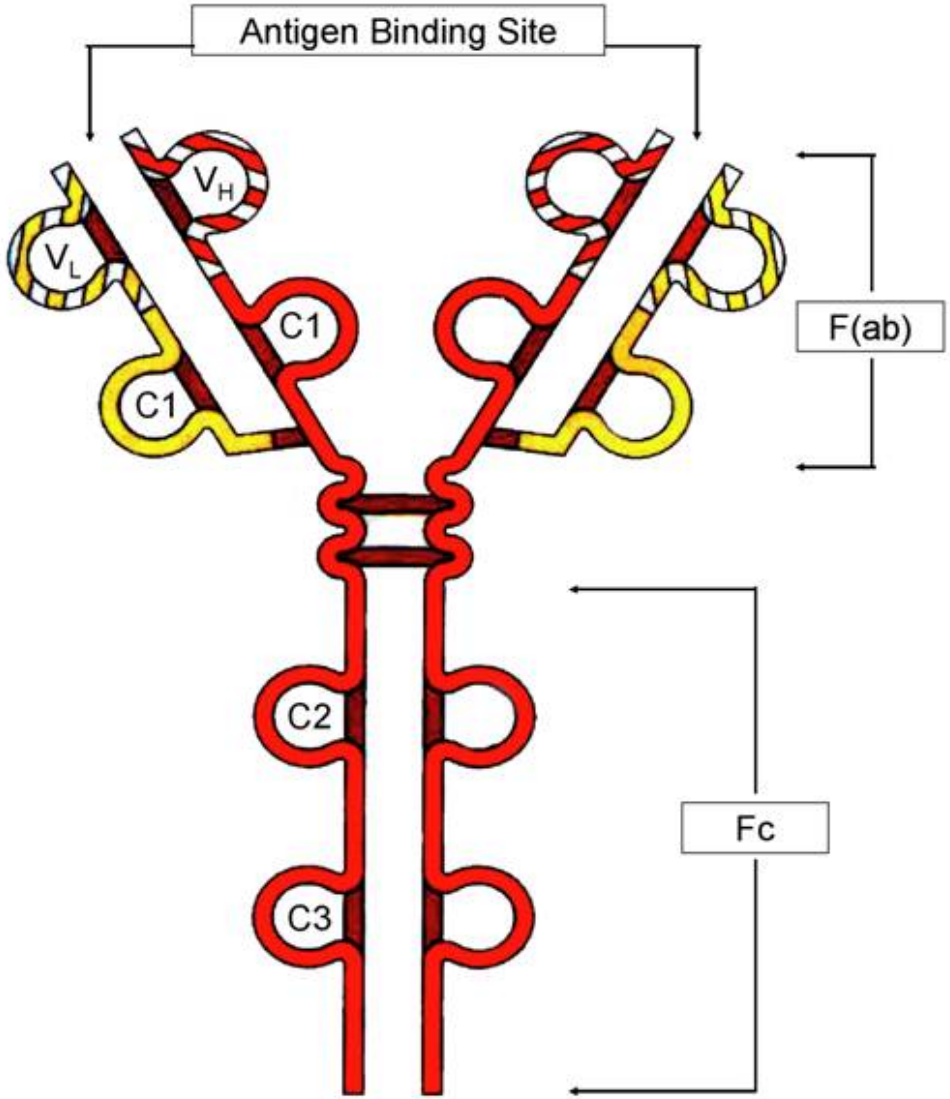

A
B
C
D
L
Signal (volts)
time, sec
a
b
c
d
Signal, delta V
[Biotin], nM
[anti-Biotin], pM
r2 = 0.995

Keys to Successful Immunosensor Development

- Inclusion of the antibodies and the sample matrix to be used in the final assay early in sensor development.
- Exhaustive characterization of the binding properties of the antibody being used in the sensor.

Other factors influencing the performance of the uranium immunosensor

- BSA, a carrier protein added to the assay system to improve antibody stability, bound the UO_2^{2+}-DCP complex with micromolar affinity (K_d of 3.3×10^{-6} M) and caused the assay to appear less sensitive for uranium.
- Covalent modification of the lysine residues of 12F6 destroyed this antibody's binding activity.
- Covalent modification of the lysine residues of 8A11 with Cy5 or Alexa 488 induced positive cooperativity in its binding to the UO_2^{2+}-DCP complex (Hill coefficient of 1.5-1.6).
- Incubation of 8A11 with saturating concentrations of fluorescently-labeled goat antibodies directed against mouse IgG increased the affinity of the native 8A11 for the UO_2^{2+}-DCP complex by three-fold.



Detailed binding studies performed for immunosensor development have revealed basic information about immunoglobulin structure and function heretofore unrecognized in the field.


2000
KIMAX

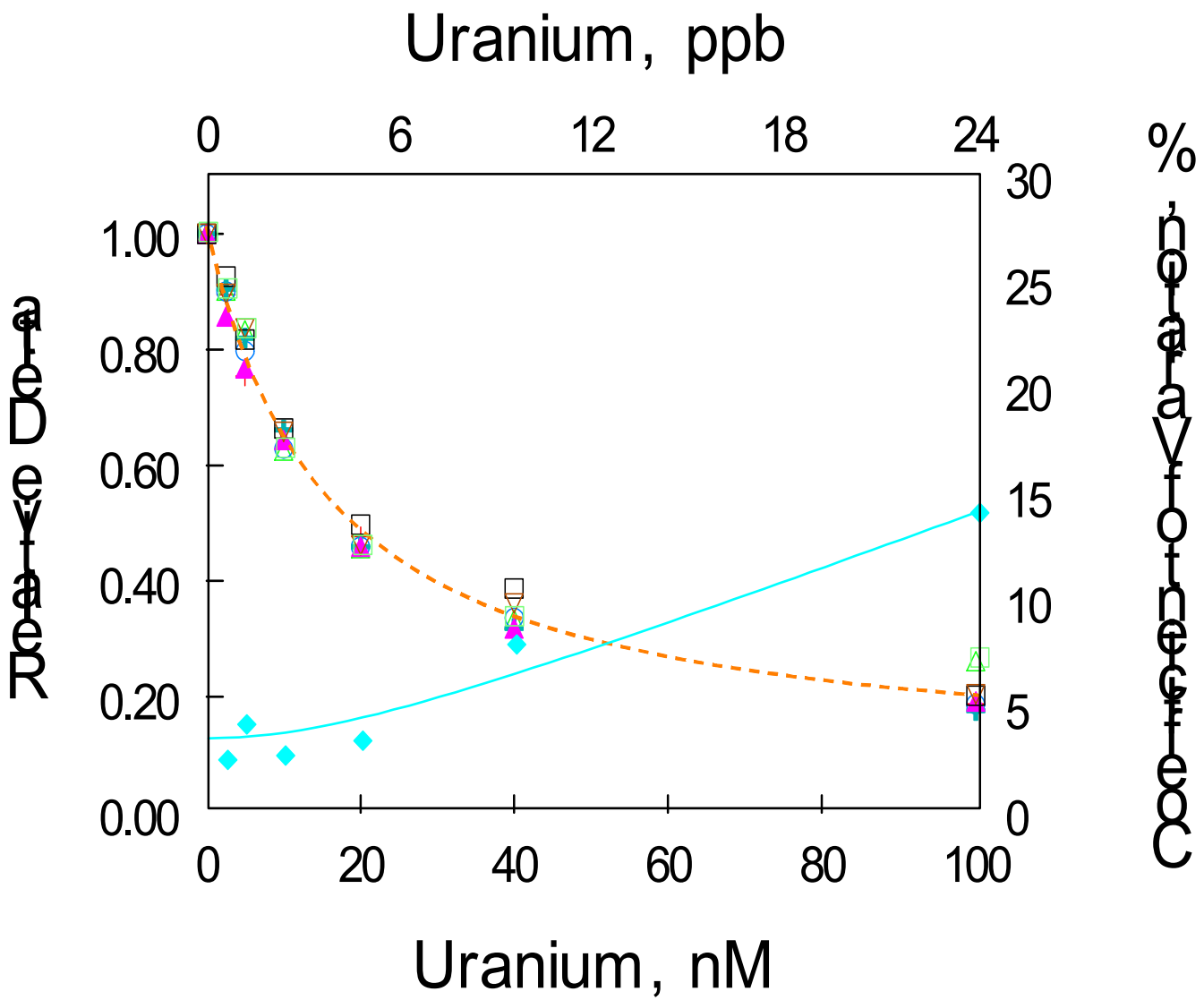
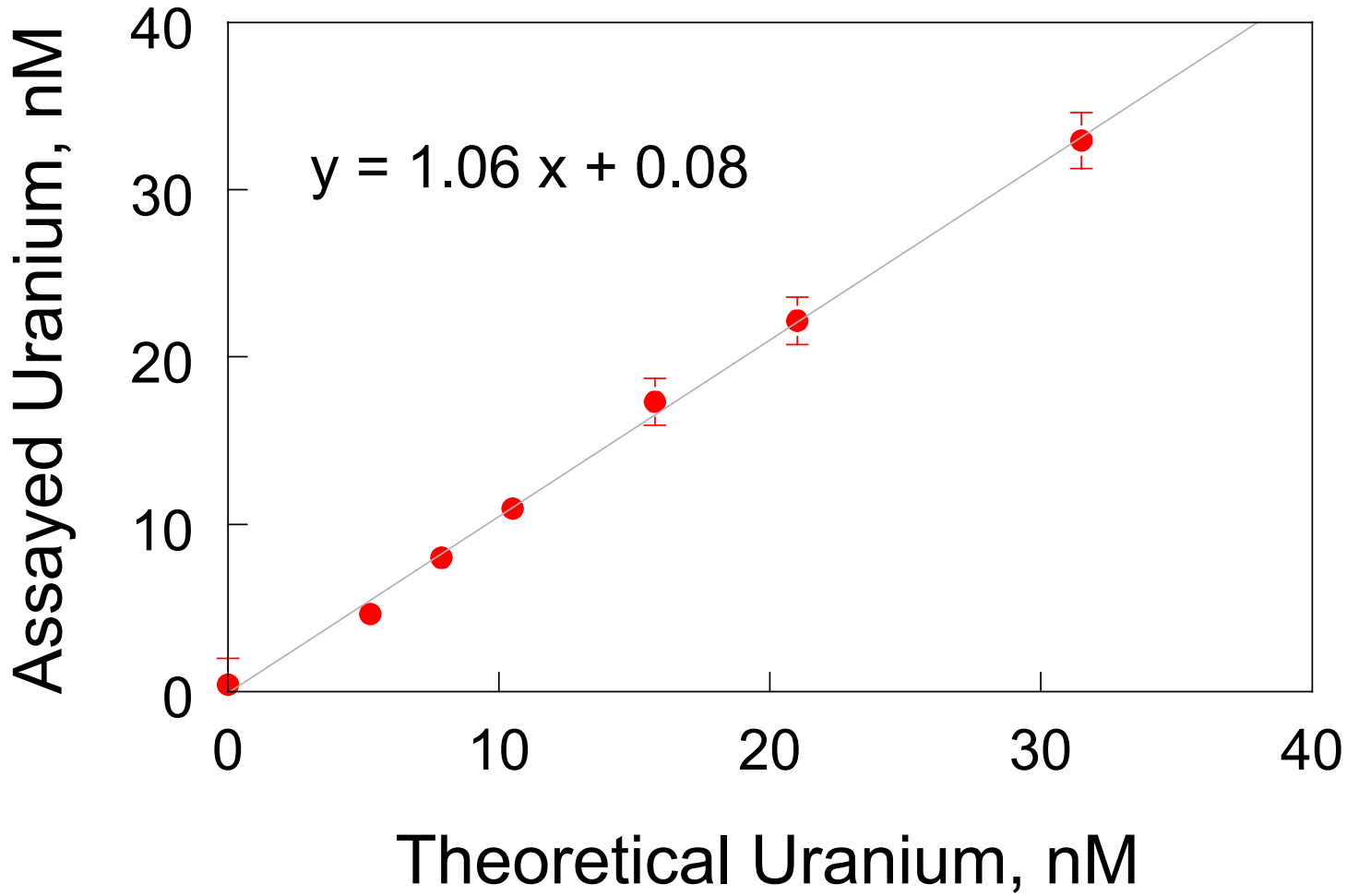

Uranium, ppb
0
6
12
18
24
1.00
0.80
0.60
0.40
0.20
0.00
30
25
20
15
10
5
0
0
20
40
60
80
100
Uranium, nM
% naïve fluorescence

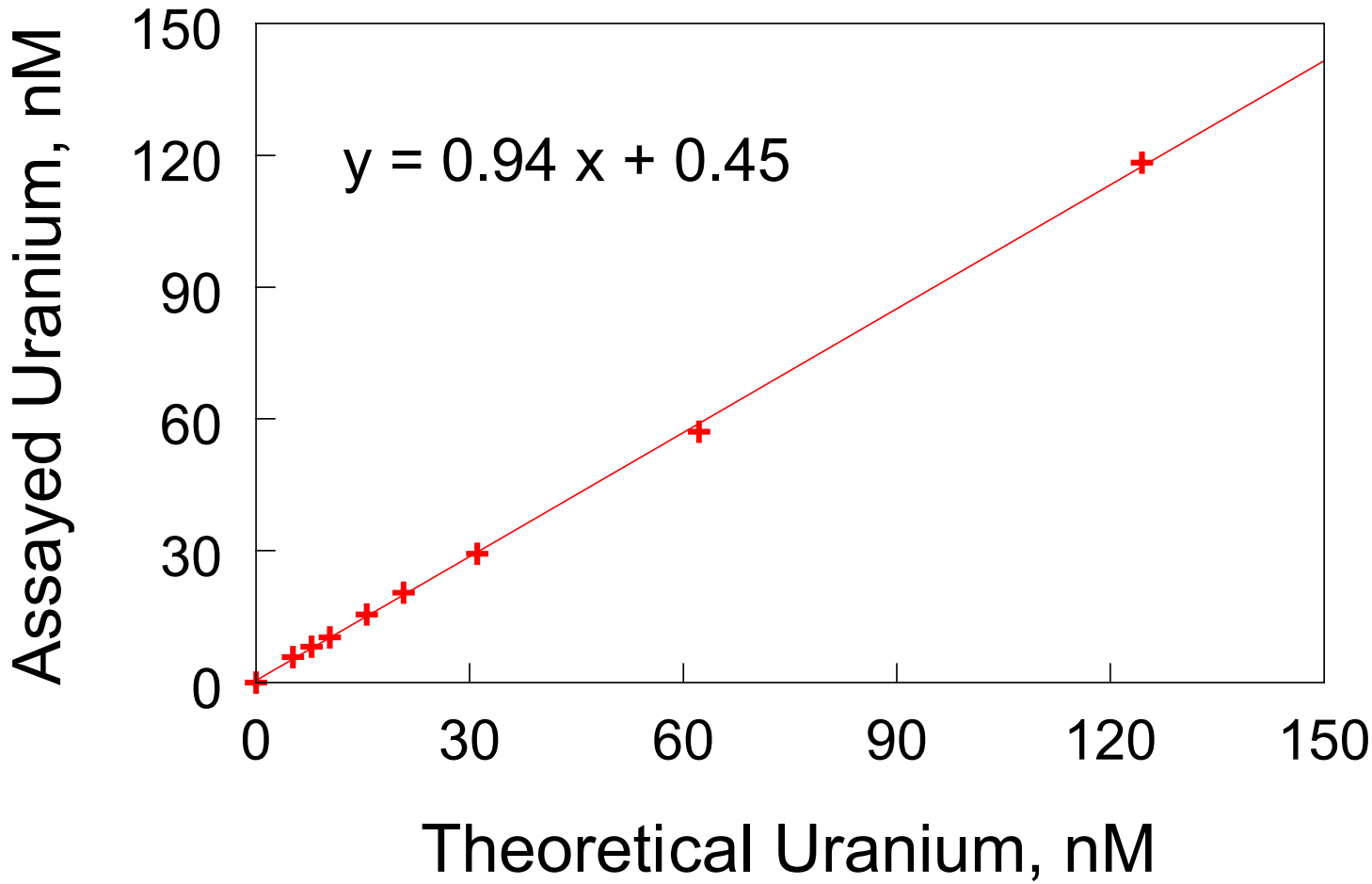
Table 1. Analytical Recovery of U(VI) added to water samples

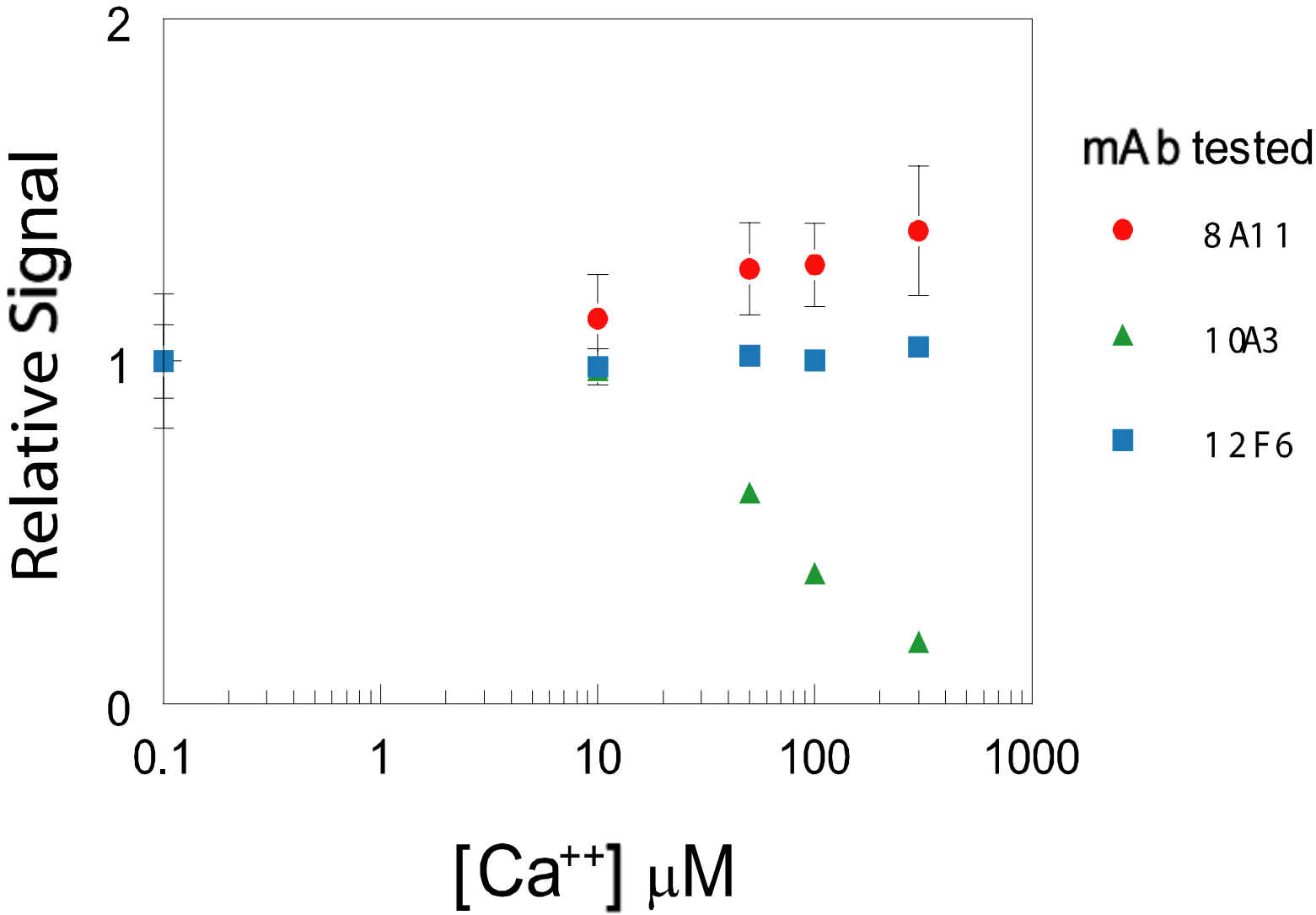
added U(VI), nM	found U(VI), nM	recovery (%)
4.0	3.107 ± 0.203	77.67 ± 6.53
7.5	7.032 ± 0.241	93.75 ± 3.43
12.5	13.008 ± 0.339	104.06 ± 2.60
15.0	15.821 ± 1.881	105.48 ± 11.9
18.0	19.064 ± 2.136	105.91 ± 11.2
20.0	21.634 ± 1.435	108.17 ± 6.63
average		**99.17 ± 7.05**

Autonomous dilution and subsequent assay of an NIST uranium standard by the in-line immunosensor

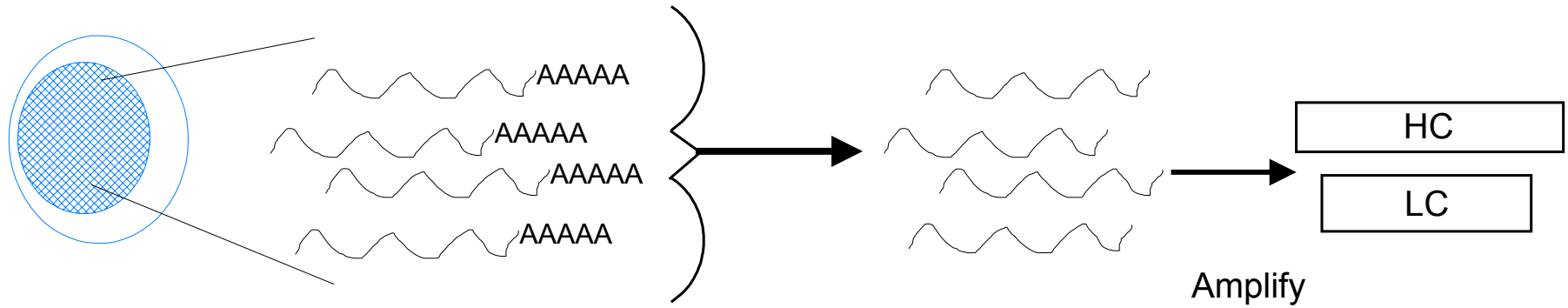


Manual dilution and subsequent assay of an NIST uranium standard with KPA




Relative Signal
2
1
0
0.1
1
10
100
1000
[Ca++] μM
mAb tested
8A11
10A3
12F6

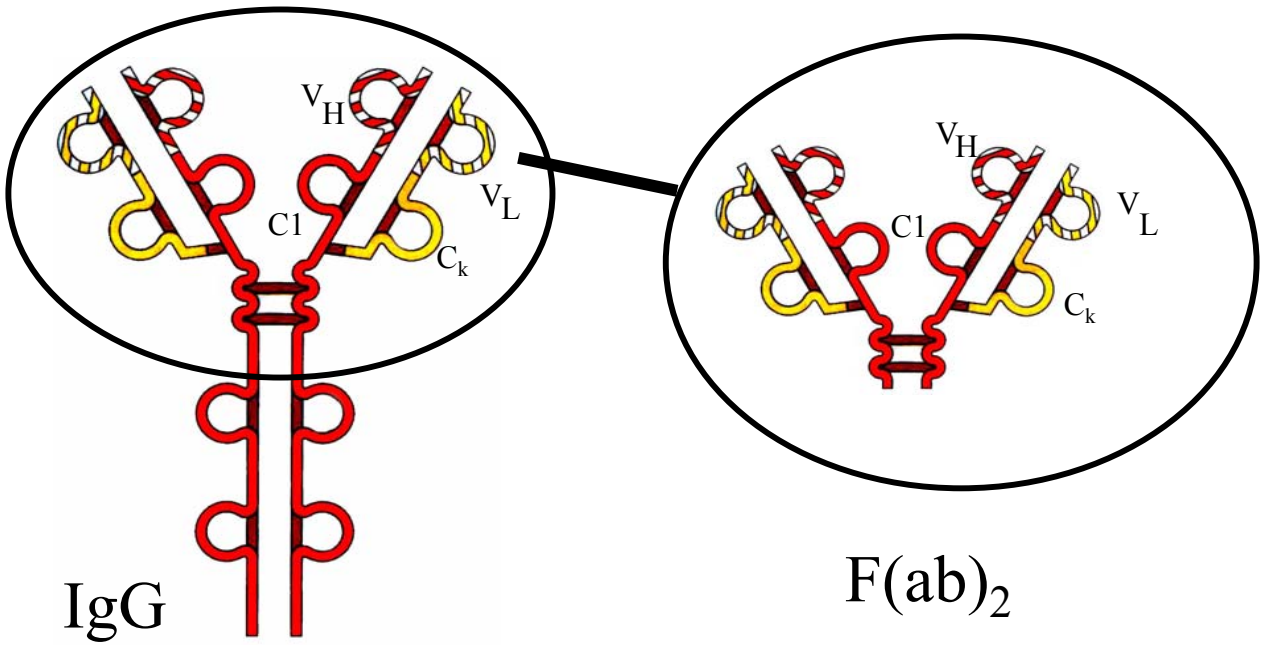
Cloning Antibody 12F6



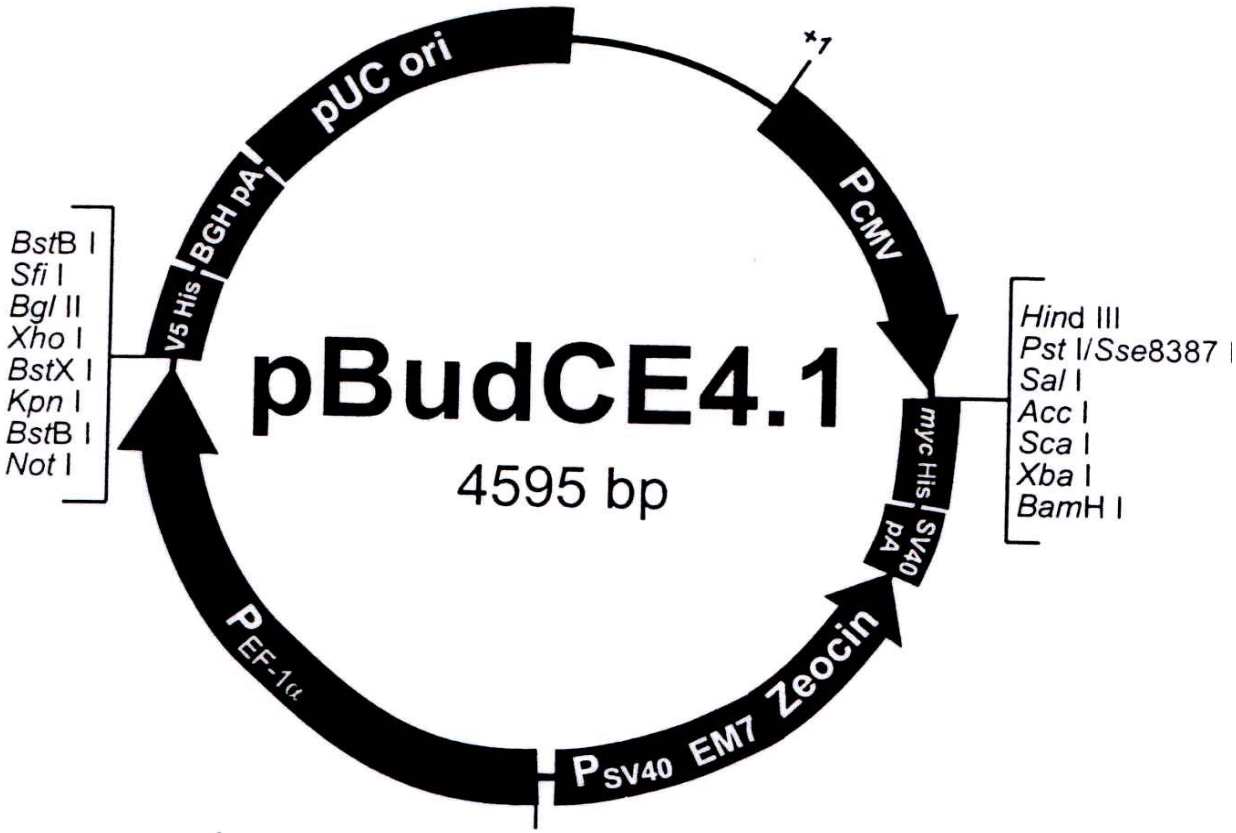
Cloning Antibody 12F6

HC-V	C1

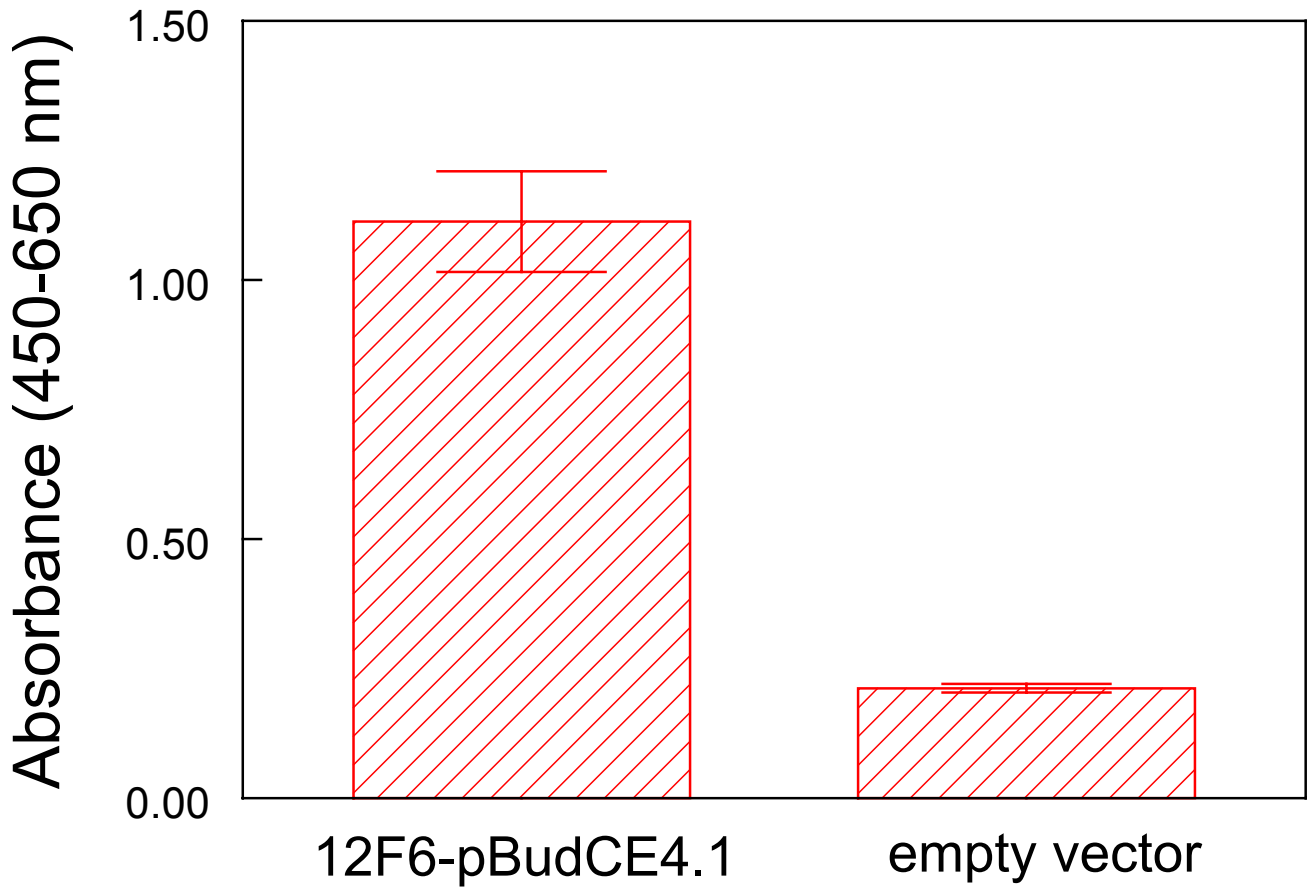
LC-V	C_K



Dicistronic vector used for recombinant antibody expression



Binding of culture supernatants from transfected cells to immobilized UO_2^{2+}-DCP

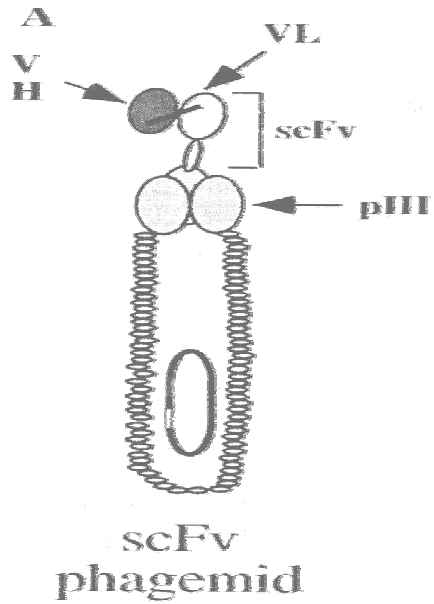


Conclusions

- Previous studies on antibody characterization and instrument development streamlined the development of an in-line immunosensor for hexavalent uranium.
- The LOD of the assay is 5.8 nM (1.38 ppb), with CV's from 2.3-13.9%.
- Expression of the 12F6 antibody as a recombinant protein will facilitate its incorporation into the sensor format.

Future Studies

- Deploy the in-line sensor at other places in the DOE complex
- Develop new assays for additional heavy metals, Hg(II) and Cr(III)
- Develop new recombinant antibody reagents based on antibody phage display techniques



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