Mount Sinai	Icahn School of Medicine at Mount Sinai	Human Immune Monitoring Core
Title		**Page 1 of 12**
Grand Serology ELISA		
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Approvals		
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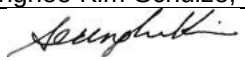		**Supersedes Date:** New

1. PURPOSE

To describe the procedures to be followed when performing Enzyme-Linked Immunosorbent Assay (ELISA) to measure antibody titers from human serum, plasma, or other soluble source for reactivity against a series of recombinant protein antigens or synthetic peptides, also known as Grand Serology. ELISA is a sensitive immunoassay that can specifically detect and quantitate the concentration of immunoglobulins (Ig) to a given antigen, and has been optimized for autoantibody profiling of tumor-associated antigens. The basic ELISA method makes use of purified recombinant proteins or synthetic peptides, which have been noncovalently adsorbed, “coated”, onto the wells of plastic microtiter plates, primarily as a result of hydrophobic interactions. The immobilized proteins/peptides serve as antigen targets for detection of antibody/antigen interactions. Following blocking of plates for any remaining adsorbing areas, immunoglobulin-containing specimens (typically serum or plasma) are placed in serial dilutions for reactivity to captured proteins, and are then detected by a second, human Ig-specific antibody (detection antibody) conjugated with an enzyme such as alkaline phosphatase (AP). This complex of antigen-serum antibody-secondary antibody with enzyme is detected by adding a fluorescent substrate for the enzyme used. The extent of fluorescence produced (arbitrary units) corresponds to the amount of antibody reactivity in the serum/plasma sample and can be conveniently measured using an ELISA-plate reader set at the appropriate wavelength. Known negative and positive control samples (human serum pools from healthy donors and cancer patients respectively) are incorporated into the ELISA on each plate in serial dilutions, to validate that the assay works as expected and to provide a reference for extrapolating titers in test samples. Grand serology ELISA will estimate the titer, i.e., the greatest dilution at which a serum or plasma sample may react to a given antigen, based on cutoffs determined from negative control. It may be necessary to perform extended dilutions on some samples to be assured that the measured ODs fall within the linear region of the titration curve.

Using ELISA as a base, multiple proteins and peptide pools can be analyzed and measured for a given number of samples.

2. MATERIALS AND EQUIPMENT

2.1 Sample to test: Plasma, serum, supernatant, spit, urine, or other sources of immunoglobulin. Recommended range of titration in this SOP optimized for serum or plasma samples.

2.2 Negative control serum pool: pre-checked pool of 3-5 healthy donor sera with no specific reactivity to tumor antigens tested and representative of average fluorescence units obtained in other healthy donor sera.

Positive control serum pool: pre-checked pool of cancer patient sera with known reactivity to antigens tested.

2.3 PBS without Ca^{2+} or Mg^{2+} (Cat. 46-013-CM 10X)

2.4 Tween 20 (Cat# BP337-500)

2.5 Fetal Bovine Serum Gemini Foundation FBS 900-108

2.6 Recombinant proteins corresponding to truncated or full-length known tumor or control antigens (purchased or provided by Nishikawa Lab, Immune Design, collaborators). Please note source of protein (E. coli, yeast, mammalian) and presence of tags (His, GST).

2.7 Optionally, synthetic long peptide pools (purchased, JPT, Biosynthesis, or Multiple Peptide Systems) covering sequences of known tumor antigens or control antigens.

2.8 Worksheet Template —attached at the end of this SOP.

2.9 Non-fat Dry Milk - Lab Scientific Cat# M0842

2.10 Secondary Antibody Goat-anti-human IgG-AP (1:3500, Biotech 2040-04) (or anti-IgA-AP (1:4000); anti-IgG1-AP (1:3000). Subclass and other isotypes also available. Recommended dilutions require re-titration with each new lot.

2.11 Substrate: AttoPhos fluorescent substrate/buffer (ref: Promega Corporation S1021)

2.12 3M Sodium Hydroxide (NaOH) (Cat# S399-500)

2.13 Microtiter Plates, high protein-binding, 96-well (Nunc Maxisorb Catalog No. 446469, or equivalent; Corning 3690)

2.14 Parafilm

2.15 Freezer (-20°C)

2.16 Pipets

2.17 Micropipets (single-channel and multichannel) and pipet tips

2.18 Refrigerator (2-8°C)

2.19 Automated Washer (405 Select TS) with Biotek Stacker (BioStack3)

2.20 ELISA Reader (BioTek NEO)

2.21 Gen5 2.01 ELISA Reader Program

2.22 Vortex

2.23 Falcon tube 15ml (polypropylene tube) (Cat#352070)

2.24 50ml Pipet Basin/reservoir (Cat#13-681-500)

3. SAFETY

3.1 Wear protective gloves and lab coat while performing this procedure. Sera and plasma are potentially dangerous and need to be handle at first in a biosafety container when dilution plates are prepared.

3.2 Perform all open-vessel work within a laminar flow biological safety cabinet to avoid contamination of samples

or operator exposure to potentially infectious materials.

3.3 Dispose of all biological waste within the appropriate waste containers.

3.4 Hydrogen peroxide is a corrosive oxidizer. Keep away from sources of ignition. Do not ingest or breath fumes. Avoid contact with skin and eyes. Never add water to hydrogen peroxide. Keep away from incompatibles such as other oxidizing agents, reducing agents, combustible materials, organic materials, metals, acids, and alkalis.

3.5 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) should be kept away from strong oxidizing agents and strong acids. Avoid contact with skin and eyes.

3.6 Sodium hydroxide is a base. It should be stored away from oxidizing agents, reducing agents, metals, acids, and alkalis. Never add water to sodium hydroxide.

4. REAGENT PREPARATION

4.1 1x PBS (Bottle C)

4.2 Wash Buffer (PBS + 0.2% Tween-20) (Bottle B)

2 L bottles of PBS 10X

17 L H_2O milliQ

Tween 20 (2 ml per liter)

4.3 Blocking Buffer

Sterile PBS plus 5% dry milk

100 ml PBS 1X

5.0 g non-fat dry milk,

4.4 Secondary Antibody

Goat anti-human IgG-AP (1:3500) **or** goat anti-human IgA-AP (1:4000)

Blocking Buffer

4.5 Substrate

AttoPhos substrate—(Ref S101C)

AttoPhos Buffer (Ref S1022)

Final concentration: 0.6 mg/ml

4.6 3M NaOH

Stopping Reaction

5. PROCEDURE

Day 1: Coating 96 well ELISA plates with desired protein or peptide pool

5.1 Prepare the proteins and peptides to coat the 96 well plates following the example below. The final protein concentration is typically 1 µg/ml and peptide at 1 µM. Some antigens may require further dilution based on observed reactivity with negative control serum pool. In conditions and instrumentation described here, fluorescence units of negative control serum pool expected between 20-250 at 1/100 dilution.

Prepare the x number of 15 ml falcon tubes with 4 ml of PBS, label them with proteins, peptide names

Add the indicated amount of proteins and peptide to the designed 15 ml tube and vortex. See example below

For an example.

- 2.5 µL of NY-ESO-1 (Nishikawa) to 4 ml of PBS
- 1.1 µL of CT10
- 4 µl of p53 pept. Pool (CI=1 mM aliquot)

Table 1: list of the antigens and Peptides and their required amounts

- NY-ESO-1 (Nishikawa)= 2.5µl
- NY-ESO-1 (Inmuno Design)=8 µl
- NY-ESO-1 (LICR)=8 µl
- P53=3.3 µl
- MAGE-A1=1 µl
- MAGE-A3=0.5 µl
- MAGE-A4=0.9 µl
- MAGE-A10=1.1 µl
- SOX2=14.3 µl
- SSX2=9.4 µl (/)
- SSX4= 2.4 µl
- CT10=1.1 µl
- CT47= 0.5 ug/ml=2.1 ul
- MELAN-A= 0.5ug/ml=0.65 µl
- HORMAD1= 0.25ug/ml 1.5 µl
- SURVIVIN=20 µl
- SURVIVIN 2B= 0.5ug/ml = 7.15 µl
- SURVIVIN Δ=4 µl
- HERV-K=8 µl
- UBTD2=6.2 µl
- XAGE=1.4 µl
- XAGE 1b=4 µl (/)
- WT1=5.1 µl
- PRAME=7.1 µl
- ERG= 0.5µg/ml = 0.8µl
- GAGE7=2 µl

Peptides pool: Final Concentration= **1µM** ; Final Volume of 1X PBS= **4ml/plate**

- NY-ESO-1 pept. Pool 1-17(CI=1 mM)=4 µl
- NY-ESO-1 pept. Pool 80-109 (CI=1 mM aliquot)=4 µl
- p53 pept. Pool (CI=1 mM aliquot)=4 µl
- CT10 pept. Pool 1-36 (CI=1 mM aliquot)=4 µl

Table 2. Protein and Peptide stock solutions kept at -80°C.

TTAs: (proteins)	*TTAs: (peptides)*
GST-HT-ESO1 (Nishikawa) 1.63mg/ml 4/4/14	NY-ESO-1 pept. Pool 1-17 4/29/15 1mM (-80C/ bottom shelf/ NY-ESO-1 pept.pool box)
NY-ESO-1 protein 0.5mg/ml IDCc002 8/17/15 MIA(IMP) 13581 Inmuno Design	NY-ESO-1 80-109 K86-2 1 10mM (-80C/ Shelf3/ Rack2/ Box11)
NY-ESO-1 protein 0.5mg/ml Manuf: 7/23/15 Lot: PBR-0039-001 LICR	p53 pept. Pool 1-30 10mM 12/9/14 (-80C/ Shelf3/ Rack4)
GST-HT-P53 1.24mg/ml 2/17/12	CT10 1-36 OLP 10mM 12/3/13 (-80C/ Bottom Shelf)
GST-HT-MAGE-A1 4mg/ml 3/9/12	
GST-HT-MAGE-A3 9.4mg/ml 2/28/12	
GST-HT-MAGE-A4 4.6mg/ml 10/27/11	
GST-HT-MAGE-A10 3.9mg.ml 2/17/12	
GST-HT-SOX2 0.28mg/ml 5/7/15	
SSX2 0.43mg/ml ph4.5 8M Urea	
GST-HT-SSX4 1.7mg/ml 2/28/12	
GST-HT-CT10 3.72mg/ml 4/14/14	
GST-HT-CT47 0.96mg/ml 2/17/12	
MELAN-A 3.2mg/ml 4M Urea ph7.5 4/22/2005 ITH	
GST-HT-HORMAD1 0.72mg/ml 3/18/14	
GST-HT-SURVIVIN 0.21mg/ml in 50% Glycerol 6/19/12	
SURVIVIN 2B 0.28mg/ml 07/10/14	
GST-HT-SURVIVIN Δ EX3 1.0mg/ml 2/8/13	
HERV-K protein 0.5mg/ml 11/19/14	
GST-HT-UBTD2 0.65mg/ml in glycerol 6/29/12	
GST-HT-XAGE 2.901mg/ml 9/8/14	
XAGE 1b Protein 1mg/ml	
WT1 Ag BMP319 0.79mg/ml Exp. 01/06/17	
ASCI a-PRAME Lot:P1206F Prep.22Jun12 0.57mg/ml (GSK Vaccines)	
GST-HT-ERG 2.665mg/ml 9/4/14	
GST-HT-GAGE7 2.22mg/ml 8/7/14	

Vortex all the 15 ml falcon tubes and transfer to the reservoir

Use repeating multichannel pipette into pre-designed location of the 1st entire 96 well plate

Repeat the 2nd protein into the 2nd entire 96 well plates

Further repeat for each protein and peptide pool used.

5.2 Incubate the plates with lids at +4°C overnight **OR** at room temperature for 2 hours

Day 1/2: Blocking the plates

5.4 Wash plates with proteins and peptides with Biotek Stack 3 machine. Select ELISA wash program, that include 3 washes with 150µl/well of 1X PBS + 0.2% Tween 20 (Buffer B - see below), and 3 washes with 150ul/well of 1X PBS

(Buffer C – see below).

Bottle B: 1X PBS + 0.2% Tween 20

Bottle C: 1X PBS

(approx.: 40 mins **or** wash in groups of 12 plates to stagger washing with coating)

5.3 Prepare Blocking Buffer (PBS + 5% dry milk). Can be prepared in advance. After mixing, place on rocker in 4°C for at least 10 mins. Blocking buffer is stable for 3 days stored at 4°C. Record the date of preparation.

For 24-26 plates:

Bottle 1: 380 ml PBS + 19 g milk

Bottle 2: 220 ml PBS + 11 g milk

5.5 Vigorously shake off plates in sink. Tap onto lined paper towels to remove any wash by-products.

5.6 Immediately add 150 µl of blocking buffer (5% dry milk/PBS) per well. Do not touch bottom of well with pipette tips.

5.7 Incubate at +4°C overnight **OR** at room temperature for 2 hours without shaking.

Day 2/3: Sample preparation, titration, and reaction

5.8 5.10 Thaw serum samples to test and to be used as controls to room temperature—O.N. at 4°C

5.11 Centrifuge sera for 5 minutes at 2000 rpm

Prepare serum dilution plate using a 4-fold dilution pattern (1:100, 1 :400, 1:1600, 1:6400) into a 2 ml 96 deep well plate,

- Add pre-determined blocking buffer to well, based on example template below for 24 antigens. (ex: 1287 µl of blocking buffer for first column corresponding to 1/100 dilution, 975 µl for other columns)
- Add pre determined amount of original serum or plasma to first column. (ex: add 13 µl of sera, making first dilution 1/100). Also place negative control serum pool in duplicate and positive control sera as per plate template (see below).
- Resuspend 1st column of 100X of diluted sera and transfer predetermined amount to next column. Do NOT resuspend.(ex: transfer 325 µl)

- Change tips and resuspend wells at 400X of diluted sera and transfer predetermined amount to next column. Do NOT resuspend.
- Change tips and resuspend wells at 1600X of diluted sera and transfer predetermined amount to next column. Do NOT resuspend.
- Change tips and resuspend well at 1600X and remove and discard predetermined amount if only 4 dilutions are tested in titration. Optionally, continue titration up to 1/10,000,000.
- Repeat for next set of samples if more than one plate per antigen is needed.
- Use worksheet prepared well in advance of assay. Add extra volume to the calculation. (Example Worksheet attached as appendix)

Transfer 325µl

Discard 325µl

	1to100	1to400	1to1600	1to6400	1to100	1to400	1to1600	1to6400	1to100	1to400	1to1600	1to6400	
	1	2	3	4	5	6	7	8	9	10	11	12	
A	HP sera 13µl + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 7 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 15 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	A
B	HP sera 13µl + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 8 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 16 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	B
C	Sample 1 13µl + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 9 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 17 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C
D	Sample 2 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 10 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 18 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	D
E	Sample 3 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 11 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C1+ + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	E
F	Sample 4 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 12 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C2+ + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	F
G	Sample 5 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 13 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C4+ + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	G
H	Sample 6 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 14 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C-(Conl) + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	H

5.19 After incubating blocked assay plates overnight at 4°C or 2 hours at room temperature, wash plates with automated washer, shake off, and tap plates on absorbent paper (as in steps 5.3 and 5.4). Immediately transfer 30ul of diluted sera from dilution plate according to worksheet. DO NOT TOUCH BOTTOM OF WELL WITH TIPS.

5.20 Incubate the assay plates for 2hrs at room temperature (or overnight at 4°C).

5.21 Wash plates using automated washer (as in steps 5.3).

5.22 Prepare secondary antibody dilution. Goat-anti-human IgG-AP (1:3500) or Goat-anti-human IgA-AP (1:4000) or Goat-anti-human IgG1-AP (1:3000) in blocking buffer. (Secondary antibody dilution may differ from lot to lot, requires titration, and use of secondary is dependent on what immunoglobulin type needs to be measured – typically IgG only).

Vortex diluted antibody. (For 24 plates, prepare 80 ml of 2nd diluted antibody) (ex: 80 ml of blocking buffer + 22.8 µl of A)

5.xx NEED STEP TO REMOVE LAST WASH HERE BY VIGOROUS SHAKING AND TAP, as in 5.4 (check number above)

5.23 Add 30 µl to secondary antibody to each well, and incubate for 60 minutes at room temperature without shaking.

5.24 Wash plates using automated washer (as insteps 5.3).

5.25 Prepare substrate. Final Concentration: 0.6 mg/ml. AttoPhos substrate/buffer REF: S1021 Promega Corporation. Do not pipet directly from buffer bottle. Use a sterile plastic spatula to weigh out substrate. (For 24 plates, weight 4.8 mg of the substrate, prepared 80 ml of buffer)

Prepare 5 ml polypropylene tubes, pour 40 ml of buffer per tube, weight 2.4 mg of the substrate, add, mix

Rock substrate/buffer combination for 10 mins at 4°C until substrate has been properly absorbed into buffer.

5.xx NEED STEP TO REMOVE LAST WASH HERE BY VIGOROUS SHAKING AND TAP, as in 5.4 (check number above)

5.26 Add 30 µl of substrate into each well of the Assay plates (and incubate in dark space for 30 minutes at room temperature without shaking.5.27 Turn on the ELISA plate reader and launch ***Gen5 2.01 Program***.

5.28 Do NOT wash assay plates after 30 mins

5.29 Add 15 µl/well of 3M NaOH (reaction stopping solution). Do NOT wash plates. During the stopping solution step, use a timer to monitor the time necessary to put the solution in all plates in order to ensure 30 minutes of incubation.

5.30 Load assay plates onto stacker being careful to load in correct numerical order as well as correct orientation for reader.

5.31 Read plates using spectrophotometer. Excitation wavelength 450mn. Emission wavelength is in the range 550-560 nm.

WorkSheet Example:

3/8/17 - 3/10/17 | 4 dilutions | 24 plates

	1to100	1to400	1to1600	1to6400	1to100	1to400	1to1600	1to6400	1to100	1to400	1to1600	1to6400	
	1	2	3	4	5	6	7	8	9	10	11	12	
A	HP sera 13µl+ 1287µl	325µl +	325µl +	325µl +	Sample 7 + 1287µl BB	325µl +	325µl +	325µl +	Sample 15 + 1287µl BB	325µl +	325µl +	325µl +	A
B	HP sera 13µl + 1287µl	325µl +	325µl +	325µl +	Sample 8 + 1287µl BB	325µl +	325µl +	325µl +	Sample 16 + 1287µl BB	325µl +	325µl +	325µl +	B
C	Sample 1 13µl +	325µl +	325µl +	325µl +	Sample 9 + 1287µl BB	325µl +	325µl +	325µl +	Sample 17 + 1287µl BB	325µl +	325µl +	325µl +	C
D	Sample 2 + 1287µl BB	325µl +	325µl +	325µl +	Sample 10 + 1287µl BB	325µl +	325µl +	325µl +	Sample 18 + 1287µl BB	325µl +	325µl +	325µl +	D
E	Sample 3 + 1287µl BB	325µl +	325µl +	325µl +	Sample 11 + 1287µl BB	325µl +	325µl +	325µl +	C1+ + 1287µl BB	325µl +	325µl +	325µl +	E
F	Sample 4 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 12 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C2+ + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	F
G	Sample 5 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	Sample 13 + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	C4+ + 1287µl BB	325µl + 975µl BB	325µl + 975µl BB	325µl + 975µl BB	G
H	Sample 6 + 1287µl BB	325µl +	325µl +	325µl +	Sample 14 + 1287µl BB	325µl +	325µl +	325µl +	C-(Conl) + 1287µl BB	325µl +	325µl +	325µl +	H

Coating in PBS 1X (Usually Cf=1µg/ml)	(30µl/well) 4C/o.n.	
Dilution plate	Vf 1300ul	

1to100	1to400	1to1600	1to6400
1287µl B.B. +	975µl B.B.	975µl B.B.	975µl B.B.
13µl sera	transfer	transfer	transfer
	325µl	325µl	325µl
2h RT			

Secondary Antibody	
Goat anti-Human IgG-AP (1:3500) 80ml B.B. + 22.8 µl AB	
1h RT	
Substrate	
80ml Attophos buffer + 48 mg powder (0.6mg/ml)	
30min RT	
Stop Reaction	
NaOH 3N 15ul/well	

*Blocking Buffer (DAY1): 380ml 1X PBS + 19 g milk (5%)

*Blocking Buffer (DAY2): 220ml 1X PBS + 11g milk (5%)

C1+	015 post (Ana's samples) 08210B-2013-015	NY-ESO-1 (Nishikawa)
		NY-ESO-1 (Immuno Design)
		NY-ESO-1 (LICR)
	positivity for His-Tag	CT10
		Melan-A
		SOX2
		SSX4
		HERV-K
	M08KEY	p53 peptide pool
C2+	010 pre (Ana's samples) 08210B-2012-010	SOX2
		ERG
		CT46
	044 pre (Ana's samples) 08210B-2013-044	CT10
C4+	F06NNO Event11 (06/06/12)	MAGE-A1
		MAGE-A3 Baculo
		MAGE-A4
		SSX2
		WT1
		PRAME
		p53 recombinant protein
	GU148.01 C1D1 Bladder cancer	p53 pool peptide
C-		Conl

Patient:

ID	GB#	Time Point	Collection Date	Month from Baseline
[redacted]		Baseline	5/14/14	0
		Month 4	8/22/14	3
		FU #1	2/20/15	9
		Baseline #2	8/7/14	0
		Month 4	11/4/14	3
		EOT	8/4/15	12
		Baseline	12/6/10	0
		Month 4	3/14/11	3
		FU #1	8/31/11	9
		FU #3	3/20/12	16
		FU #5	12/28/12	25
		Baseline	5/8/13	0
		Month 4	8/6/13	3
		Baseline	8/1/12	0
		Month 4	10/26/12	3
		EOT	3/18/13	8
		FU #2	8/22/13	13
		FU #4	2/21/14	19

[redacted] 24 plates 3/10/17

Plate #	TAA	[]	PBS 1X	Vol. of TAA
1	NYESO1 (N)	1µg/ml	4ml	2.5µl
2	NYESO1 (ID)	1µg/ml	4ml	8µl
3	NYESO1 (LICR)	1µg/ml	4ml	8µl
4	P53	1µg/ml	4ml	3.3µl
5	MAGE-A1	1µg/ml	4ml	1µl
6	MAGE-A3 (B)	1µg/ml	4ml	13.6µl
7	MAGE-A4	1µg/ml	4ml	0.9µl
8	MAGE-A10	1µg/ml	4ml	1.1µl
9	SOX2	1µg/ml	4ml	14.3µl
10	SSX4	1µg/ml	4ml	2.4µl
11	CT10	1µg/ml	4ml	1.1µl
12	CT47	***0.5µg/ml***	4ml	2.1µl
13	MELAN-A	***0.5µg/ml***	4ml	0.65µl
14	HORMAD1	***0.25µg/ml***	4ml	1.5µl
15	SSX2	1µg/ml	4ml	6.15µlL
16	XAGE-1b	1µg/ml	4ml	1.43µl
17	SURVIVIN-DELTA	1µg/ml	4ml	4µl
18	UBTD2	1µg/ml	4ml	6.2µl
19	XAGE	1µg/ml	4ml	1.4µl
20	WT1	1µg/ml	4ml	5.1µl
21	PRAME	*1µg/ml*	4ml	7.1µl
22	ERG	***0.5µg/ml***	4ml	0.8µl
23	GAGE7	1µg/ml	4ml	2µl
24	DHFR	1µg/ml	4ml	20.4µl

	TTAs:
1	GST-HT-ESO1 (Nishikawa) 1.63mg/ml 4/4/14
2	NY-ESO-1 protein 0.5mg/ml IDCc002 8/17/15 MIA(IMP) 13581 Inmuno Design
3	NY-ESO-1 Protein 0.5mg/ml Manuf: 7/23/15 Lot:PBR-0039-001-LICR
4	GST-HT-P531 1.24mg/ml 2/17/12
5	GST-HT-MAGE-A1 4mg/ml 3/9/12
6	GST-HT-MAGE-A3 Baculo 294 ug/ml 01/18/02
7	GST-HT-MAGE-A4 4.6mg/ml 10/27/11
8	GST-HT-MAGE-A10 3.9mg.ml 2/17/12
9	GST-HT-SOX2 0.28mg/ml 5/7/15
10	GST-HT-SSX4 1.7mg/ml 2/28/12
11	GST-HT-CT10 3.72mg/ml 4/14/14
12	GST-HT-CT47 0.96mg/ml 2/17/12
13	MELAN-A 3.2mg/ml 4M Urea ph7.5 4/22/2005 ITH
14	GST-HT-HORMAD1 0.72mg/ml 3/18/14
15	SSX2 0.65mg/ml
16	XAGE1b 2.823mg/ml
17	GST-HT-SURVIVIN Δ EX3 1.0mg/ml 2/8/13
18	GST-HT-UBTD2 0.65mg/ml in glycerol 6/29/12
19	GST-HT-XAGE 2.901mg/ml 9/8/14
20	WT1 Ag BMP319 0.79mg/ml Exp. 01/06/17
21	ASCI a-PRAME Lot:P1206F Prep.22Jun12 0.57mg/ml (GSK Vaccines)
22	GST-HT-ERG 2.665mg/ml 9/4/14
23	GST-HT-GAGE7 2.22mg/ml 8/7/14
24	DHFR- 0.196ng/mL

Definition of Seroconversion (defined as a ≥ 4-fold increase over baseline titer). Reciprocal titers will be extrapolated and considered significant if >100. In samples with multiple serum collection time points available, significant changes in humoral response will be defined as antigen-specific antibody going from undetectable (titer <100) to detectable (titer >100), or with titers at least 4-fold different between time points (Gnjatic, Old, & Chen, 2009).

References:

Gnjatic, S., Old, L. J., & Chen, Y. T. (2009). Autoantibodies against cancer antigens. *Methods Mol Biol, 520*, 11-19. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19381944. doi:10.1007/978-1-60327-811-9_2