

# 1/8/10 B6 B8 Whole cell KA,CTL 48hr

Friday, January 08, 2010

9:57 AM

From B6 and B10 samples

B6-(K1-K4 & C1-C2)

B8-(K5 & C3-C5)

Added 1mL PBS (+20uL PIC) to each tube

Dounce (pestle B) ~8 times

Spin 800g/5min

Aspirate supernatant (pellets were ~60-80uL volume)

Add 250uL RIPA (+5uL PIC) to K1, K2, C1, C2 (roughly 3x the cell pellet volume)

Vortex

5min sonicator (High 30sec on/off)

Add 500uL RIPA (+10uL PIC) to K3, K4, C3, C4

Vortex

5min sonicator (High 30sec on/off)

Add 750uL RIPA (+15uL PIC) to K5, C5

Vortex

5min sonicator (High 30sec on/off)

Spin max/10min

(Pellets for K1, K2, C1, C2 were bigger than pellets for the other two groups)

Aliquot 3x~80uL, 3sets stored in -80C in three bags

## Bradford Assay

0, 1, 2, 4, 8, 12µL of 1mg/mL BSA standard (duplicates)

Samples 2µL (singlets)

Add Sample or Standard

Add 500µL water

Add 500µL Bradford Reagent

Pipette up and down to mix ~6x

Measure at 595nm (Let sit at RT at least 5min, let Spectrophotometer warm up at least 15min)

[file:KAvsCTL-InVivo/WB-B6,B8\(wholecell\)--48hr\(P10\),KA,CTL/010810BradfordAssay.xlsx](file:KAvsCTL-InVivo/WB-B6,B8(wholecell)--48hr(P10),KA,CTL/010810BradfordAssay.xlsx)

# Western Blot 1/2 Samples KA, CTL

Friday, January 15, 2010  
5:17 PM

20 $\mu$ L total + 4 $\mu$ L 6x Laemmli Buffer (B160 freezer in Shawn's box)

\*Rainbow marker found in Amy's box in B160 freezer\*

GEL 1

Well #		Sample ( $\mu$ L)	H <sub>2</sub> O ( $\mu$ L)	
1	Marker	5		
2	C1	7.63070	12.3693	
3	K1 (1/4)	1.9513828	18.0486172	
4	K1	7.8055312	12.1944688	
5	C3	12.12629	7.87371	
6	K3 (1/4)	3.035368068	16.96463193	
7	K3	12.14147227	7.85852773	
8	C5	17.30225289	2.697747111	
9	K5	16.56183173	3.438168265	
10	K5 (1/4)	4.140457934	15.85954207	

## Running Gel Electrophoresis

Assemble gel in gel rig (**4-12%** Tris-Glycine gradient gels)

--Mix, spin, 10min ~65°C-70°C, RT 10+ min, spin

--Load samples into their appropriate wells ~21 $\mu$ L

Run gel (80V/30min) then (130V/1hr)

## Membrane Transfer

Cut a piece of PVDF membrane (Amersham Hybons-P)--found in the drawer just below the WB area (10cm x 7.5cm)

Cut the upper right corner of the membrane

**ONLY HANDLE THE MEMBRANE WITH FORCEPS**

Wet membrane for 30 sec in methanol, 5min in water, at least 10min in 1x Transfer buffer

Let gel equilibrate in 1x Transfer Buffer at least 10min

Assemble 'sandwich' for Bio-Rad's Transblot under the Transfer Buffer solution to avoid bubbles and ripping of the gel

Pre-wet sponges, filter papers (slightly bigger than gel) in pre chilled 1x Transfer Buffer

**Black - Sponge - FP- gel - membrane - FP - sponge**

(Marker on right, upper cut corner on left) [file:#Stuff/WBTransfer.avi](#)



Transfer for **1hr05min at 100V** - OR - overnight at 30V

Using forceps, transfer membrane(cut down membrane around gel) to tray filled with PBS-T

Rinse briefly in PBS-T

Immerse membrane in Blocking buffer,

cover with parafilm, Block at **RT for 2 hours.**

(Blocking Buffer: 5.0g of 10% non-fat dry milk in 50 mL [1x PBS - 0.01% Tween 20])

**Notes: When running the gel and using about 700mL of Running Buffer, more "Running Buffer" was made by mistakenly diluting 10X PBS to 1XPBS and added to the gel setup. The samples concentrated on top but faded on the bottom and but the marker was fine. The samples concentrated into a line at the last 10 minutes of the run.**

# Western Blot 2/2 Samples KA, CTL

Friday, January 15, 2010  
9:34 AM

Save milk from incubation and put aside in designated plastic tubes (found in WB box in B156) to be used for secondary antibody and primary antibodies.

Soak/rinse membranes in PBS-T

Use the tubes from the Western Blot box in B156 refrigerator to prepare all of the needed primary and secondary antibodies:

--after delivering the antibody to its appropriate tube, pipette up and down to mix.

**\*After using, mark the antibody tubes before placing back in freezer.**

--seal the tubes and invert them to homogenize the antibody solution.

--prepare two small blue trays.

--place each membrane face up into its appropriate tray and pour the corresponding primary antibody solution over each membrane.

Incubate with primary antibody diluted in PBS-T **for 5 hours**

**G9a ab 1:25,000 (2.5 $\mu$ L/10mL of 5% milk) (make a 1:9 dilution and use 2.5  $\mu$ L of the diluted amount in 10mL of solution)**

**coREST ab 1:5000 (2 $\mu$ L/10mL of 5% milk)**

Both abs found in QQ Lab Freezer

Pour out the contents of the tray and rinse once with 0.01% Tween 20 in 1xPBS

Wash 3x5min with 0.01% Tween 20 in 1xPBS

Incubate with secondary antibody diluted in 5% Blocking buffer for **1hr** at RT  
(1:10000 dilution for secondary antibody)

**G9a Anti-rabbit** (QQ fridge secondary antibody box; small glass container with a black cap) :  
1 $\mu$ L /10mL-->(5mL 10% non-fat dry milk solution of before + 5mL PBS-T)

Use the same milk solution in which the membrane was incubating overnight but dilute with PBS-T.

**coREST Anti-mouse** (QQ fridge secondary antibody box; small glass container with a black cap) : 1 $\mu$ L /10mL-->(5mL 10% non-fat dry milk solution of before + 5mL PBS-T)

Use the same milk solution in which the membrane was incubating overnight but dilute with PBS-T.

Pour out the contents of the tray and rinse once with 0.01% Tween 20 in 1xPBS

Wash 4x15min with 0.01% Tween 20 in 1xPBS

Detect with Amersham ECL

#### Procedure for Development:

1.First add 4mL to a glass tube using a dispenser during the washes for the detection mixture to be at room temperature.

2.Add the 100 $\mu$ L right before use.

3.Pick up the membranes with forceps and shake off as much liquid as possible.

4.Set both membranes face up, side by side in a small tray with G9a on top and TNF-alpha on the bottom.

5.Use a dispenser to pour the detecting solution over the 2 membranes and let the membranes equilibrate for 5 min.

6.Cut a piece of plastic wrap and lay it flat on the bench top.

7.Use forceps to lay both membranes face down on the plastic wrap (NRSF top and Actin bottom); push the membranes together to line them as one and push out bubbles using forceps.

8.Fold the plastic wrap and tape all of the back. Stick the enveloped membrane to the lower right corner of the exposure chamber.

#### In the Dark Room:

1.Fill the baths with their appropriate solutions (developer, DI water, fixer).

2.Only use the top safely light and pull out film from the orange Western Blot box in the upper right cupboard.

3.Without exposing the film to light, place it in the exposure chamber for a specific amount of time. Once the time is done, flip over the film and expose it for the other specific amount of time.

4.At the end of the second exposure, engulf the film into the developer for 1 minute, into DI water for 1 second, and into fixer for at least 2 minutes.

5.Check the development on the film and determine quantities of other times accordingly.

6.Rinse the developed film with DI water and hang it to dry for later analysis.

# FS Rats 1/11/10 2 months

Monday, January 11, 2010

5:12 PM

**Resect hippocampus \*\*Left and Right in Separate tubes**

Sac'd: 1/11/10

1 FS, 2CTL-Males B-10/31 FS-11/10 +62days

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