Carbonyl Western blot protocol

		settings	time
1	Gel running (just after DNPH reactions with reagents from Oxyblot Protein Oxidation Detection Kit from Millipore Corporation, # S7150). Gels (4-20% Linear gradient Tris-HCl gel (8.5 x 13.5 cm)) are purchased from Bio-Rad, electrophoresis is performed in Bio-Rad Criterion cell in 1xTris/Glycine/SDS buffer (1xTGS). We are running two identical gels – one is for Carbonyl assay and second one is for Coomassie staining (with Bio-Safe Coomassie stain from Bio-Rad, # 161-0786).	195 - 200 v	50-55 min
2	- Soaking of gel for Carbonyl in Towbin transfer buffer, - Soaking of Bio-Rad NC Membrane (8.5 x 13.5 cm) and Extra Thick Blot Paper in Towbin transfer buffer. <u>Towbin transfer buffer:</u> Dissolve 3.03 g Tris and 14.4 g Glycine in 800 ml H_2O, and add 200 ml methanol.		20 min
3	Transferring in Towbin transfer buffer in Bio-Rad Semi-Dry Electrophoretic Transfer Cell with PowerPacHC Power Supply	20 v – constant For default: set max 3A set max 300 wt	40 min
4	Rinsing briefly in 1xPBS and storage (wet) at this step	Store at +4^{o}C	overnight
5	Blocking in 35 ml 1xPBS + 0.05% Tween 20 + 1% BSA (Blocking/Dilution solution).	~ 50-70 rpm	1 hour
6	Incubation with first antibody: 20 ml Blocking/Dilution solution +133 µl 1^{o}A a/b	~ 50-70 rpm,	1 hour
7	Washing in 1xPBS + 0.05% Tween 20.	**~ 150 rpm:** - 20 ml - 30 ml **~ 100 rpm** - 30 ml - 30 ml - 40 ml	 rinse rinse 15 min 5 min 5 min
8	Incubation with second antibody: 20 ml Blocking/Dilution solution +67 µl 2^{o}A a/b	~ 50-70 rpm	1 hour
9	Washing in 1xPBS + 0.05% Tween 20 as after first a/b.		
10	Developing with SuperSignal West Pico Chemiluminescent Substrate (Pierce, # 34080).	10 ml of Stable Peroxide solution +10 ml of Luminol/Enhancer solution	5 min
11	Exposure with films (Blue Lite Autorad film ISC Bio Express, # F-9024) and image with CCD camera, FujiFilm Las1000.		For film: 10 sec, 30 sec, 1 min