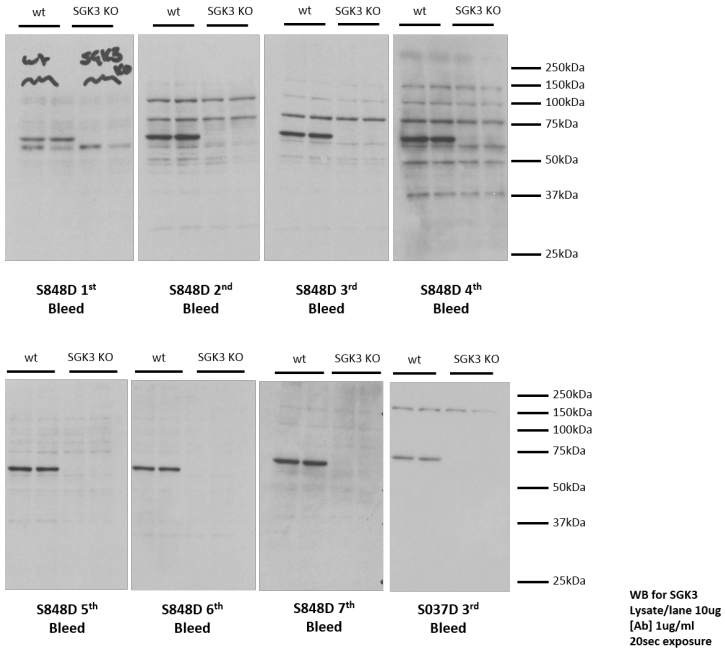
Anti-SGK3 Ab S848D

1. Testing 10 Bleeds of the new S848D anti-SGK3 Ab for Immunoblotting from Cell Lysate

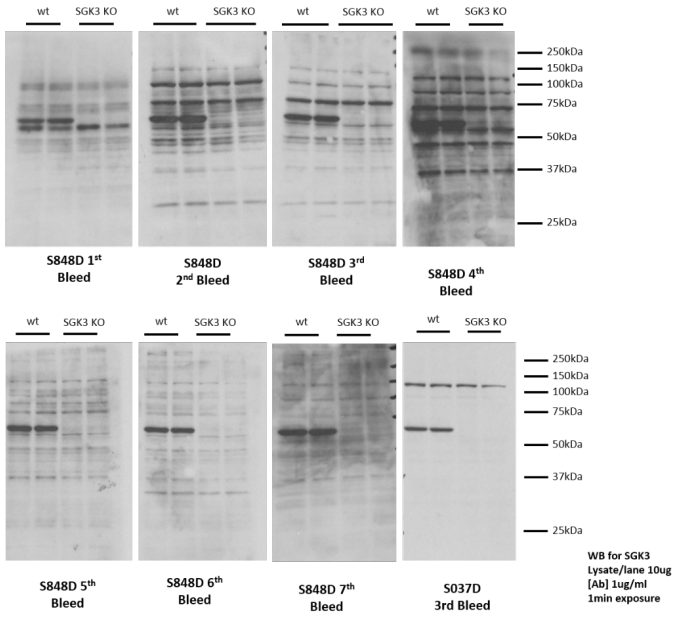
Cell Line: HEK293 and HEK293 SGK3 KO
Lysate/lane: 10ug
[Ab]: 1ug/ml

Wildtype HEK293 and SGK3-KO HEK293 cell lysates were immunoblotted for SGK3, using the each of the first 7 Bleeds of the S848D antibody, and the currently used S037D 3rd Bleed. 10ug whole cell lysate was loaded onto a 10% SDS-PAGE gel, and membranes probed with 1ug/ml primary antibody, and 1:2500 secondary α-Sheep antibody. Membranes were developed using ECL Chemiluminescent Reagent, and simultaneously exposed for 20 seconds (short exposure) or 1 minute (long exposure) onto light-sensitive films.

a) Short exposure



b) Long exposure



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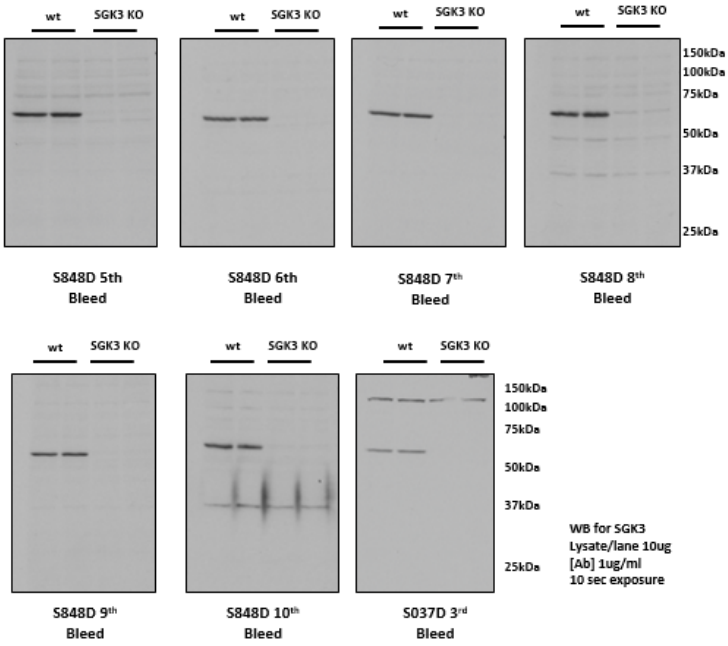
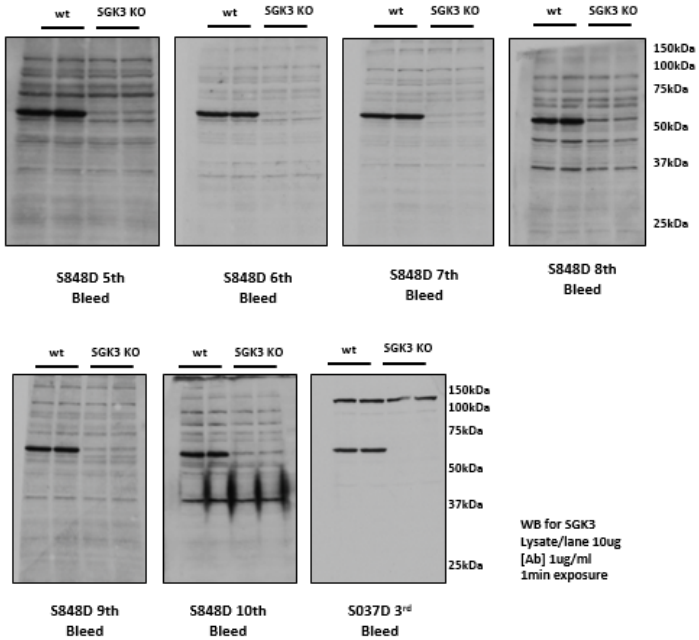
On the basis of this data, S848D Bleed 6 appears to be the best antibody for Immunoblotting. However, all of the later bleeds 5-7 were significantly better than the early antibodies, so the antibody project was continued up to bleed 10 and these antibodies compared to the same protocol. Membranes were simultaneously exposed for 10 seconds (short exposure) or 1 minute (long exposure).

c) Short exposure



d) Long exposure



Bleed 6 again appears to show the best specificity for SGK3, with the later bleeds starting to show more non-specific binding. Bleeds 7 and 9 however also produce a strong signal for SGK3.

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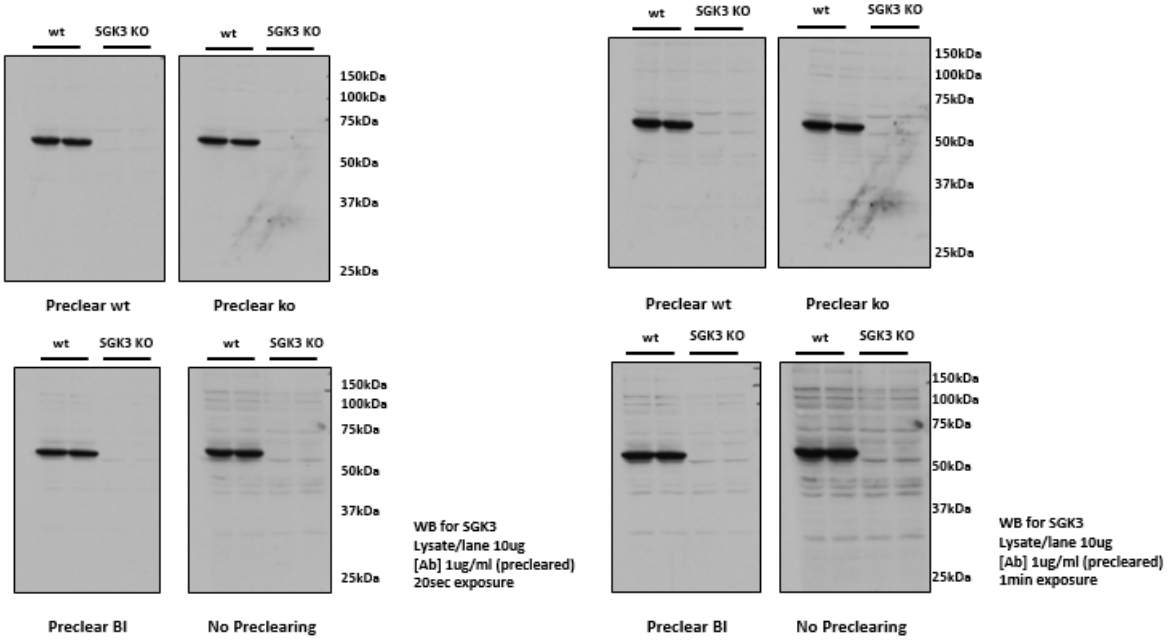
2. **Preclearing S848D Bleed 6 against saturated membranes.**

Cell Line: HEK293 and HEK293 SGK3 KO
Preclearing 2x12hr
Lysate/lane: 200ug
Immunoblot
Lysate/lane: 10ug
[Ab]: 1ug/ml

From the above experiments, Bleed 6 of the S848D antibody was by far the best, but still showed some non-specificity. To attempt to reduce non-specific recognition, 1ug/ml S848D Bleed 6 was precleared against either blank membranes or membranes saturated with either wt or SGK3 KO whole cell lysate (200ug lysate/lane) for 2x12hrs and compared to the non-precleared antibody. The antibodies were then used to immunoblot against HEK293 and SGK3-KO HEK293 cell lysates to the same protocol described above.



Based on the above data, preclearing the antibody against just a blank membrane appears to drastically improve its specificity, however saturation of the membrane with either wt or SGK KO whole cell lysate does not appear to have any additional effect.

For Immunoblotting SGK3 from whole cell lysate, S848D Bleed 6 antibody can be used at 1ug/ml concentration. It is also recommended to preclear the antibody first for at least 24 hours on a Nitrocellulose Membrane. S848D Bleeds 7 and 9 could also be used but have slightly increased non-specific targets.

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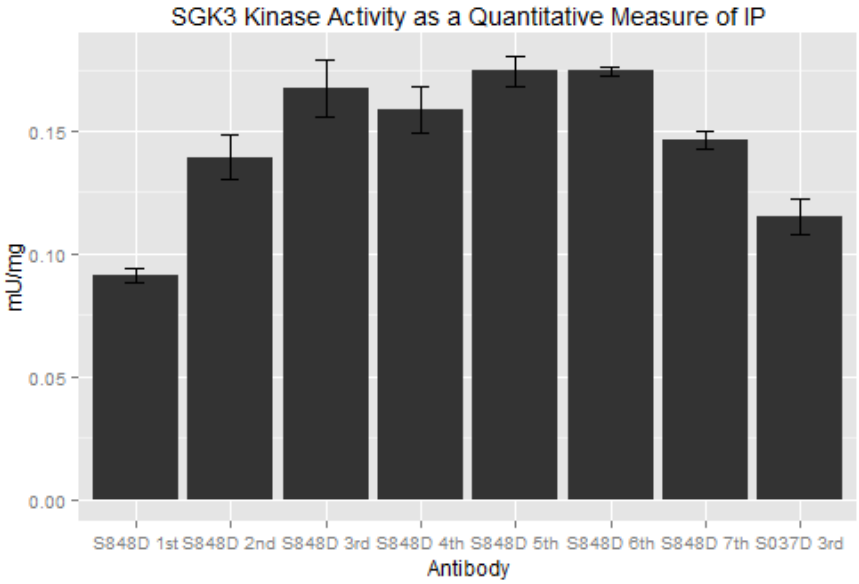
3. **Testing 7 Bleeds of the new S848D anti-SGK3 Ab for Immunoprecipitation of SGK3 from Cell Lysate.**

Cell Line: HEK293
Lysate/IP: 3mg
Ab/IP: 3ug

3mg of HEK293 whole cell lysate underwent immunoprecipitation for SGK3 using 3ug of each Ab, coupled to 6ul Sepharose G beads. After IP, Kinase Activity Assay was performed using Crosstide substrate and ^{32}P ATP for 30 minutes. Raw CPMs were normalised using similarly coupled PreImmune IgG as a negative control.



Protein was eluted from beads in 90ul 2X LDS with 5% BME, to give a 100ul sample. 10ul of this sample was loaded onto a 10% SDS-PAGE gel, with 20ug whole cell lysate and IP supernatant. Immunoblotting was performed using Anti-SGK Ab from Sigma (Cat# S5188)

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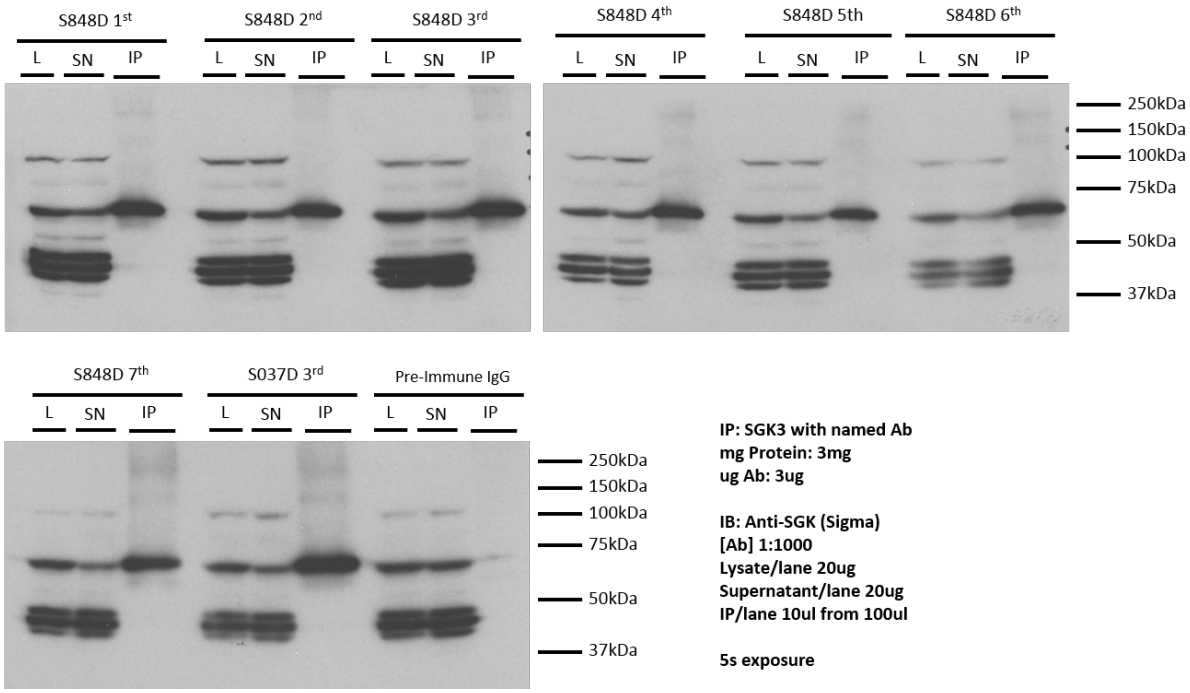

The data suggest that all the antibodies IP SGK3 from whole cell lysate very well. The later bleeds 5-7 also show more substantial depletion from the supernatant.

S848D is suitable for immunoprecipitation of SGK3 from whole cell lysate, at a ratio of 1ug Ab to 1mg lysate. S848D Bleed 6 appears to be optimal, however all of bleeds 5-7 appear to IP the protein well.