

TECHNICAL SERVICE GUIDE: Western Blotting

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing?
2. What size bands were expected and what size bands were detected?
3. Was the blot blank or was a dark background or non-specific bands seen?
4. Did this same vial of product work in the past? What were the results?
5. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLES AND CONTROLS

1. From what species (animal) was the sample and what type of sample was used (cell lysate, tissue, purified protein, etc.)? What lysis buffer was used?
2. How much sample was loaded on the gel?
3. If the blocking peptide was used, was it used as a negative control?
4. What positive and/or negative controls were used, and what were the results?

BLOCKING

1. What were the blocking conditions (blocking solution, how long, what temperature, etc.)?

PRIMARY AND SECONDARY ANTIBODIES

1. At what dilution(s) was the primary antibody used and what was the diluent?
2. What were the incubation conditions (time, temperature) for the primary antibody?
3. From which company was the secondary antibody obtained and what type of secondary was being used (HRP or AP conjugated, anti-rabbit or anti-goat, etc.)?
4. What detection system was used, and was it compatible with the secondary conjugate?
5. At what dilution(s) was the secondary antibody used and what was the diluent?
6. What were the incubation conditions (time, temperature) for the secondary antibody?
7. Has a secondary control been performed successfully?
8. If using Cruz Markers, how many μL were loaded, and was a Cruz Marker compatible secondary antibody used?
9. Can you verify that all Molecular Weight Markers transferred to the membrane?
10. At what temperature has the primary antibody been stored? Has it ever been frozen?

MEMBRANE, GEL, DETECTION

1. What detection system was used and what were the exposure times for the film?
2. What type of membrane was used (PVDF or nitrocellulose)?
3. What percentage gel was used? Was this appropriate for the size of the protein?

TECHNICAL SERVICE GUIDE: Immunoprecipitation

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing?
2. Did this same vial of product work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLES

1. From which species (animal) was the sample and what type was used (cell lysate, tissue, purified protein, etc.)? What lysis buffer was used?
2. How many cells were lysed or what was the protein concentration and volume of the sample that was used in the immunoprecipitation?

PRE-CLEARING

1. Was a pre-clear step done prior to adding primary antibody?
2. What species of immunoglobulin (rabbit, goat, etc.) was used for the pre-clear step?
3. What protein agarose-conjugate was used in the pre-clear step (Protein-A, Protein-G Agarose, etc.)?

ANTIBODIES AND IMMUNOPRECIPITATION REAGENTS

1. How many micrograms of primary antibody were added to the total cell lysate?
2. Was the primary antibody directly conjugated to agarose?
3. How long was the primary incubated with the total cell lysate?
4. What immunoprecipitation reagent was used (Protein-A Agarose, Protein-G Agarose, etc.)?
5. At what temperature has the primary antibody been stored? Has it ever been frozen?

WASHES AND SPINS

1. Is the final wash done with a stringent reagent (RIPA) or a mild reagent (PBS)?
2. How fast were the samples spun (in RPM)?

DETECTION

1. What detection method was used (Western Blotting or Radioactive labeling)?
2. If Western Blot was performed, what antibody was used? Was this the same as the antibody used in the immunoprecipitation reaction?
3. If Western Blot was performed, what size bands were expected and what were detected?
4. If Western Blot was performed, were heavy (55 kDa) and light (27 kDa) bands detected?

CONTROLS

1. Has the antibody (used in the IP) been used successfully in any other applications with the sample?
2. What positive and/or negative controls were done and what were the results?

TECHNICAL SERVICE GUIDE: Immunohistochemistry**Catalog #:****Lot #:****PROBLEM AND PREVIOUS EXPERIENCE**

1. What is the specific problem you are experiencing?
2. Did this same vial of product work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

STAINING PATTERN

1. What pattern of staining was expected and what was observed?

CONTROLS

1. Has the primary antibody (used in the immunohistochemistry) been used successfully in any other applications (Western Blot, Immunoprecipitation, Immunohistochemistry with different method of fixation) with the same sample?
2. What positive and/or negative controls were done and what were the results?

SAMPLES AND FIXATION

1. From which species (animal) was the sample?
2. What type of sample was stained (frozen tissue, paraffin-embedded tissue, tissue culture cells)?
3. What method of fixation was used (acetone, formalin, methanol)?
4. Was Antigen-Retrieval performed? If so, by what method (heat treatment, enzyme digestion)?

BLOCKING

1. What were the blocking conditions (blocking solution, species of serum, length of incubation)?

PRIMARY ANTIBODY

1. At what dilution(s) was the primary antibody used and what was the diluent?
2. What were the incubation conditions (time, temperature) for the primary antibody?
3. At what temperature has the primary antibody been stored? Has it ever been frozen?

SECONDARY ANTIBODY

1. From which company was the secondary antibody obtained and what type of secondary was being used (goat anti-rabbit, rabbit anti-mouse, etc.) and what secondary antibody conjugate was used (biotin, FITC, Rhodamine, Texas Red)?
2. At what dilution(s) was the secondary antibody used and what was the diluent?

DETECTION

1. What method of detection was used (ABC Kit, ImmunoCruz Kit, Immunofluorescence)?
2. If a kit was used, was the kit used successfully with other primary antibodies?
3. If performing immunofluorescence, what was the time period between staining and visualization?
4. If performing immunofluorescence, was the filter used appropriate for the fluorochrome?

TECHNICAL SERVICE GUIDE: Flow Cytometry (Single Color)

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing?
2. Did this same vial of product work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE PREPARATION

1. What sample preparation protocol are you using and what anticoagulant are you using?
2. From what species (human or mouse) was the sample?
3. What type of sample was used (cultured cells, tissue or blood)?
4. How much time has passed since this sample was collected?
5. What lysing solution are you using?
6. How many cells are you using per test?
7. Do you need to activate your cells? If so, what were the conditions?
8. How much time passed between the preparation of the cells and data acquisition? How were the prepared cells stored?

BLOCKING

1. If using mouse cells, are you using a blocking reagent? If so, what reagent are you using?

STAINING

1. What percentage of cells and what cell type should this antibody stain?
2. On what population of cells are you gating? What does your SSC vs. fluorescence plot look like?
3. How intense is the staining (use the scale: 10E0-10E1: negative; On 10E1 line: weak positive; 10E1-10E2: Intensity of +1; 10E2-10E3: Intensity of +2; 10E3-10E4: Intensity of +3)?

CONTROLS

1. Are you using a positive control? Does this positive control stain all of the leucocytes, as expected?
2. Are you using the correct fluorochrome-conjugated isotype-specific control IgG? Is it negative, as expected?

PRIMARY ANTIBODY

1. Was the antibody diluted? If so, by what factors was the antibody titrated (ie. 1:10, 1:50, etc.)?
2. What incubation conditions are you using (30 minutes on ice or at 4° C in the dark)?

STORAGE CONDITIONS

1. How has the antibody been stored? Has the antibody ever been frozen?

TECHNICAL SERVICE GUIDE: EMSA

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing?
2. Is the TransCruz Gel Supershift form of the product (the "X" form) being used?
3. Did this same vial of product work in the past? What were the results?
4. Did other lots of this product work in the past? Which lots? What were the results?

CONTROLS

1. What was used as a positive control and what were the results?
2. Was a mutant oligonucleotide sequence used as a negative control? If so, what were the results?

SAMPLE

1. From what species (animal) and cell line was the nuclear extract derived?
2. Were the cells induced or treated? If so, how?
3. How many micrograms of nuclear extract were used and what was the total reaction volume?
4. What was the reaction buffer?

OLIGONUCLEOTIDE

1. Which oligonucleotide was used and how much oligonucleotide was added to the nuclear extract?
2. How long was the oligonucleotide incubated with the nuclear extract?
3. What labeling kit was used to label the probe?

PRIMARY ANTIBODY

1. How many microliters of TransCruz Gel Supershift primary antibody were added to the reaction?
2. How long was the primary antibody incubated with the nuclear extract?
3. Has the antibody been used successfully in any other application(s) with the same nuclear extract?

ORDER OF ADDITION

1. Which order of addition did your experiment follow:
 - a. Nuclear Extract + Oligonucleotide + Antibody
 - b. Nuclear Extract + Antibody + Oligonucleotide

SHIFT

1. In the absence of antibody, is protein binding to the oligonucleotide causing a shift?
2. After adding antibody, what was observed (a Shift in the band size, no Shift in the band size, a complete Block of the signal or a Partial Block, with a less intense signal)?

GEL

1. How many micrograms of protein were loaded on the gel?

TECHNICAL SERVICE GUIDE: siRNA

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing?
2. Did this same vial of siRNA work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE

1. Into what cell line and species (animal) was the siRNA transfected?
2. If your cells are adherent, what is the confluency of the transfected culture (in %)?
3. If your cells are a suspension culture, how many cells were used?
4. Were the cells induced or treated? If so, how?
5. What is the half-life of the protein to be knocked down (in hours)?

TRANSFECTION

1. Which transfection medium was used? Which transfection reagent?
2. What were the transfection incubation conditions (time, temp, siRNA concentration)?
3. How many rounds of transfection were performed before processing the cells?
4. If multiple rounds were used, how long was each incubation?
5. What was the transfection efficiency (in %)?

VISUALIZING RESULTS

1. How have you visualized your results (Western blot or Quantitative RT-PCR)?
2. If Western blot, which control antibody are you using in order to visualize the knockdown?
3. What were your results in WB?
4. If Quantitative RT-PCR, which primers were used?
5. Have the primers proven to be effective in the past?
6. How have you verified the purity of your RNA preparation?
7. What were your cycling temperatures (annealing, extension)?

TECHNICAL SERVICE GUIDE: shRNA Plasmid

Catalog #:

Lot #:

EXPERIMENTAL RESULT

1. What is the specific problem you are experiencing with the shRNA (i.e. no knockdown, cytotoxicity, off-targeting)?
2. Did the same vial of shRNA work in the past? What were the results?
3. Did other lots of this shRNA work in the past? Which lot(s)? What were the results?
3. How did you measure knockdown (Western blot, RT-PCR)?
 - 3a. If you performed Western blot, what were the results?
 - 3b. If you performed quantitative RT-PCR, which primers were used?
 - Have the primers proven to be effective in the past?
 - What were your cycling temperatures (annealing, extension)?
4. Please attach an image of your results

SAMPLE

1. Into what cell line and species (animal) was the shRNA transfected?
2. What cell type was used (primary, immortal, stem)?
3. If your cells are adherent, what is the confluency of the transfected culture (in %)?
3. If your cells are a suspended culture, how many cells were used?
4. Were the cells induced or treated? If so, how?
5. What is the half-life of the protein to be knocked down (in hours)?

TRANSFECTION

1. Which transfection medium was used? Which transfection reagent was used?
2. What were the transfection incubation conditions (time, temp, shRNA concentration)?
3. What time points after transduction was knockdown measured?

PUROMYCIN

1. What concentration of puromycin (sc-108071) was applied to the cells?

CONTROLS

1. Have you assayed transfection efficiency using our copGFP control plasmid (sc-108083)?
2. Have you performed a negative control using a control shRNA plasmid (sc-108060, sc-108065, sc-108066)?

STORAGE

1. At what temperature has the shRNA been stored?

TECHNICAL SERVICE GUIDE: shRNA Lentiviral

Catalog #:

Lot #:

EXPERIMENTAL RESULT

1. What is the specific problem you are experiencing with the Lentiviral Particles (i.e., no knockdown, cytotoxicity, off-targeting)?
2. Did the same vial of Lentiviral Particles work in the past? What were the results?
3. Did other lots of this Lentiviral Particles work in the past? Which lot(s)? What were the results?
3. How did you measure knockdown (Western blot, RT-PCR)?
 - 3a. If you performed Western blot, what were the results?
 - 3b. If you performed quantitative RT-PCR, which primers were used?
 - Have the primers proven to be effective in the past?
 - What were your cycling temperatures (annealing, extension)?
4. Please attach an image of your results

SAMPLE

1. Into what cell line and species (animal) were the Lentiviral Particles transfected?
2. What cell type was used (primary, immortal, stem)?

TRANSDUCTION

1. What MOI(s) were tested? $MOI = \frac{\text{Total \# cells per well}}{\text{Total uL of particles per well} \times 5e6}$
2. What concentration of Polybrene was added to the medium?
3. At what time points after transduction was knockdown measured?

PUROMYCIN

1. What concentration of puromycin (sc-108071) was applied to the cells?

CONTROLS

1. Have you assayed transfection efficiency using our copGFP control Lentiviral Particles (sc-108084)?
2. Have you performed a negative control using our control shRNA Lentiviral Particles (sc-108080)?

STORAGE

1. At what temperature have the Lentiviral Particles been stored?

TECHNICAL SERVICE GUIDE: CRISPR KO and HDR Plasmid

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing? (i.e., no knockdown, no GFP/RFP expression, cell death)?
2. Did this same vial of KO or HDR work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE

1. What type of cells are you transfecting?
Cell line and species:
Type of cell used (primary, immortal, stem):
2. Have you successfully transfected this cell line before? If so, what was the transfection efficiency?
3. How many cells were seeded?
4. If your cells are adherent, what was the confluency of the transfected culture?

TRANSFECTION

1. What method was used for transfection (transfection reagent, electroporation)?
2. Which transfection medium was used? Which transfection reagent was used?
3. How much plasmid DNA and transfection reagent did you use?
4. At what time point did you visually confirm transfection?
5. What was the transfection efficiency?

PUROMYCIN (IF HDR PLASMID WAS CO-TRANSFECTED WITH KO PLASMID)

1. At what time point was puromycin applied to the cells?
2. What concentration of puromycin (sc-108071) was applied to the cells?

EXPERIMENTAL RESULT

1. How did you measure knockdown (Western blot, PCR)?
2. At what time point after transfection or selection did you measure knockdown?
3. If you performed Western blot:
 - a. What were the results?
 - b. Which control antibody did you use to visualize knockdown?
4. If you performed PCR:
 - a. What were the results?
 - b. Have the primers proven to be effective in the past?
5. Please attach image(s)

CONTROLS

1. Have you performed a negative control using our Control CRISPR/Cas9 Plasmid (sc-418922)? If so, what were the results?

STORAGE

1. At what temperature has the plasmid(s) been stored?

TECHNICAL SERVICE GUIDE: CRE Vector

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing? (i.e., RFP expression, puromycin resistance, cell death)?
2. Did this same vial of Cre work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE

1. What type of HDR transfected cells are you using?
Cell line and species:
Type of cell used (primary, immortal, stem):
2. How did you confirm successful HDR integration (i.e., RFP expression and/or puromycin selection)?
3. How many cells were seeded?
4. If your cells are adherent, what was the confluency of the transfected culture?

TRANSFECTION

1. What method was used for transfection (transfection reagent, electroporation)?
2. Which transfection medium was used? Which transfection reagent was used?
3. How much Cre Vector and transfection reagent did you use?

EXPERIMENTAL RESULT

1. How did you verify that HDR Plasmid DNA was excised (Western blot, PCR, RFP expression, puromycin selection)?
2. At what time point after transfection did you verify knockout?
3. If you performed Western blot:
 - a. What were the results?
 - b. Which control antibody did you use to visualize knockdown?
4. If you performed PCR:
 - a. What were the results?
 - b. Have the primers proven to be effective in the past?
5. If you performed RFP visualization:
 - a. What were the results?
 - b. How did you screen for RFP expression?
6. If you performed puromycin selection:
 - a. What were the results?
 - b. At what time point was puromycin applied to the cells?
 - c. What concentration of puromycin (sc-108071) was applied to the cells?
7. Please attach image(s)

STORAGE

1. At what temperature has the Cre Vector been stored?

TECHNICAL SERVICE GUIDE: CRISPR Activation Plasmid

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing? (i.e., no activation, cell death)?
2. Did this same vial of ACT work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE

1. What type of cells are you transfecting?
Cell line and species:
Type of cell used (primary, immortal, stem):
2. Have you successfully transfected this cell line before? If so, what was the transfection efficiency?
3. How many cells were seeded?
4. If your cells are adherent, what was the confluency of the transfected culture?

TRANSFECTION

1. What method was used for transfection (transfection reagent, electroporation)?
2. Which transfection medium was used? Which transfection reagent was used?
3. How much plasmid DNA and transfection reagent did you use?
4. At what time point did you visually confirm transfection?
5. What was the transfection efficiency?

ANTIBIOTIC SELECTION

1. If you selected using antibiotics:
 - a. Which antibiotics were used?
 - b. How much of each antibiotic was added?
 - c. How long were cells subject to antibiotic treatment?

EXPERIMENTAL RESULT

1. How did you measure activation (Western blot, PCR)?
2. At what time point after transfection did you measure activation?
3. If you performed Western blot:
 - a. What were the results?
 - b. Which control antibody did you use to visualize activation?
4. If you performed PCR:
 - a. What were the results?
 - b. Have the primers proven to be effective in the past?
5. Please attach image(s)

CONTROLS

1. Have you performed a negative control using our Control CRISPR Activation Plasmid (sc-437275)? If so, what were the results?

STORAGE

1. At what temperature has the plasmid been stored?

TECHNICAL SERVICE GUIDE: Lentiviral Activation Particles

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing? (i.e., no activation, cell death)?
2. Did this same vial of LAC work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE

1. What type of cells are you transducing?
Cell line and species:
Type of cell used (primary, immortal, stem):
2. Have you successfully transduced this cell line before?
3. How many cells were seeded?
4. If your cells are adherent, what was the confluency of the transduced culture?

TRANSDUCTION

1. What concentration of Polybrene® (sc-134220) was added to the medium?
2. How much lentiviral was added?

ANTIBIOTIC SELECTION

1. If you selected using antibiotics:
 - a. Which antibiotics were used?
 - b. How much of each antibiotic was added?
 - c. How long were cells subject to antibiotic treatment?

EXPERIMENTAL RESULT

1. How did you measure activation (Western blot, PCR)?
2. At what time point after transduction did you measure activation?
3. If you performed Western blot:
 - a. What were the results?
 - b. Which control antibody did you use to visualize activation?
4. If you performed PCR:
 - a. What were the results?
 - b. Have the primers proven to be effective in the past?
5. Please attach image(s)

CONTROLS

1. Have you performed a negative control using our Control Lentiviral Activation Particles (sc-437282)? If so, what were the results?

STORAGE

1. At what temperature has the plasmid been stored?
2. How many freeze-thaw cycles has the product been through?

TECHNICAL SERVICE GUIDE: Double Nickase Plasmid

Catalog #:

Lot #:

PROBLEM AND PREVIOUS EXPERIENCE

1. What is the specific problem you are experiencing? (i.e., no knockdown, no GFP expression, cell death)?
2. Did this same vial of NIC work in the past? What were the results?
3. Did other lots of this product work in the past? Which lots? What were the results?

SAMPLE

1. What type of cells are you transfecting?
Cell line and species:
Type of cell used (primary, immortal, stem):
2. Have you successfully transfected this cell line before? If so, what was the transfection efficiency?
3. How many cells were seeded?
4. If your cells are adherent, what was the confluency of the transfected culture?

TRANSFECTION

1. What method was used for transfection (transfection reagent, electroporation)?
2. Which transfection medium was used? Which transfection reagent was used?
3. How much plasmid DNA and transfection reagent did you use?
4. At what time point did you visually confirm transfection?
5. What was the transfection efficiency?

PUROMYCIN

1. At what time point was puromycin applied to the cells?
2. What concentration of puromycin (sc-108071) was applied to the cells?

EXPERIMENTAL RESULT

1. How did you measure knockdown (Western blot, PCR)?
2. At what time point after transfection or selection did you measure knockdown?
3. If you performed Western blot:
 - a. What were the results?
 - b. Which control antibody did you use to visualize knockdown?
4. If you performed PCR:
 - a. What were the results?
 - b. Have the primers proven to be effective in the past?
5. Please attach image(s)

CONTROLS

1. Have you performed a negative control using our Control Double Nickase Plasmid (sc-437281)? If so, what were the results?

STORAGE

1. At what temperature has the plasmid(s) been stored?