ColonyFinder® Immunoscreening Kit

Description

ColonyFinder Immunoscreening Kit 69283-3

The ColonyFinder Immunoscreening Kit is designed for detecting proteins expressed in bacterial colonies with mouse antibodies. The method is very rapid and sensitive; positive clones can be identified within 3 hours of beginning the procedure. The kit contains critical components needed for screening up to fifty 82mm colony lift filters:

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Components

- 125ml Colony Denaturing Solution (20mM Tris-HCl pH 7.9, 6M urea, 0.5M NaCl)
- 40ml 10% Gelatin
- 35ml 20% Tween 20
- 40µl Goat Anti-mouse IgG Alkaline Phosphatase Conjugate
- 20ml 20X Alkaline Phosphatase Buffer
- 1.5ml BCIP Color Development Substrate (42mg/ml in dimethylformamide)
- 1.5ml NBT Color Development Substrate (83mg/ml in 70% dimethylformamide)
- Positive Control HSV•Tag™ Antibody
- Positive Control Clone glycerol stock

Colony Screening by Protein Expression

General Considerations

The number of clones required to achieve a given probability that a given sequence will be present in a gene library is N = ln(1-P)/ln(1-1/n), where N = the number of clones required, P= the probability desired (*e.g.* 0.99), and 1/n = the fractional proportion of the total sequence represented by target sequence (Sambrook *et al.* (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor). In apSCREEN™ library that will be screened by expression of target DNA, this number must be multiplied by a factor of 6 to account for 3 possible reading frames and 2 possible orientations.

For example, if apSCREEN library having an average insert size of 100bp is prepared from a 2000bp cDNA clone, [ln(1-.99)/ln(1-100/2000)] × 6 = 540 recombinants would need to be screened for a single epitope. In practice several plates having 1-2000 colonies each provide more than enough recombinants for screening with a given antibody.

In contrast, to scan a bacterial genome (3×10^7 bp) for epitopes with a library having an average insert size of 100bp would require screening 8.4×10^6 clones. In this case it would be more practical to construct and screen an initial library having larger inserts, followed by the creation of secondary libraries with smaller inserts from positives isolated from the first library. For example, if the initial library had an average insert size of 1500bp, 5.5×10^5 clones would need to be screened. This number is more feasible for the construction and screening procedures.

Immunoscreening

When using this system to detect epitopes is it important to note that in most cases only contiguous epitopes will be expressed. Conformation-dependent epitopes consisting of different protein domains will probably not be formed unless they are encoded on a single DNA fragment and fold properly in the bacterial host. Therefore, not all antibodies are suitable for mapping with this technique (or any other peptide fragmentation or synthetic method). Signal to noise ratios obtained with this procedure depend on several factors. First, the amount of antigen that is deposited on the filter varies with each fusion protein based on its expression level and stability in the host cells. (This system tends to minimize variability by producing a strongly expressed, stable gene *10* fusion protein.) Second, the quality (titer and affinity) of the screening anti-

body is of obvious importance. Generally, antibodies that produce good signals on Western blots are likely to produce good results in immunoscreening. Like conformation-dependent antibodies, those directed against carbohydrate determinants are not suitable for immunoscreening. Third, crude antisera and ascites occasionally contain IgG components that bind to *E. coli* proteins that can produce high backgrounds on colony lifts. The effect of these factors can be minimized by using the least amount of antibody that still gives a strong signal with a given amount of antigen and gives little reactivity with *E. coli* proteins. Various antibody dilutions can be easily tested by a dot blot assay using known amounts of antigen in parallel with similar amounts of *E. coli* extract (prepared by sonicating *E. coli* to break the cells and centrifuging to remove cell debris). In general, dilutions from 1:200 to 1:10,000 are appropriate for antisera, ascites fluids, or purified antibodies, whereas hybridoma tissue culture supernatants usually require dilutions from 1:10 to 1:100.

If non-specific background due to cross-reactivity with filter-bound *E. coli* proteins remains to be a problem, the antibody preparation can be preabsorbed with an *E. coli* lysate. As a starting point, incubate 1mg/ml extract with antisera diluted 1:1000 for 30 minutes prior to incubating the antisera with filters. The amount of extract and the antibody dilution can be varied to optimize signal to noise ratios.

The recommended colony immunoscreening procedure is described below. This procedure can be modified for screening with other ligands, and the conditions may need to be optimized for particular applications. For this purpose it is useful (when feasible) to clone the entire target sequence into the pSCREEN vector such that the domain of interest is expressed, and use this clone as a positive control in the optimization experiments.

The T7 RNA polymerase carried by host strain NovaBlue(DE3) drives the expression of fusion proteins from pSCREEN plasmids. Under most conditions these proteins accumulate as insoluble inclusion bodies. Although the T7 RNA polymerase is under *lacUV5* control, substantial amounts of protein are produced even in the absence of IPTG induction. Inclusion bodies appear to provide the advantage of sequestering expressed proteins so that they are less susceptible to proteolytic breakdown, and so that potentially harmful products do not affect cell viability.

1. Plate transformants in NovaBlue(DE3) at the desired density (up to 10,000 on an 82mm plate; usually 1-2000 is recommended) on LB plates containing 50μg/ml carbenicillin and 15μg/ml tetracycline. The approximate number of colonies obtained per μl of transformation should be known from initial plating experiments. We recommend screening fresh transformants to minimize the probability of biasing the library due to disproportionate growth rates of individual clones during amplification. Colonies are ready for screening even when small (0.5-1mm in diameter), this size is usually reached within 17-18hr at 37°C. Chill plates at 4°C for one-half hour prior to making colony lifts so that the agar will not stick to the nitrocellulose.
2. Carefully overlay the plates with nitrocellulose filters (*e.g.* Schleicher and Schuell BA85). Wear gloves and handle the filters by the edges. Bend slightly and allow the center to touch the plate first. Filters may be marked by poking an 18 gauge needle in 3 asymmetric places into the filter and plate. If desired, needles may be dipped into waterproof ink before stabbing into the filter and plate. Number both the filter and the plate. After one minute of contact, carefully peel the filter off of the plate. Colonies should stick to the filter; however, we have observed that sufficient protein is deposited so that strong signals are still produced even if colonies do not remain on the filter. Plates may be returned to 37°C for several hours to regenerate colonies.
3. Lyse the colonies by putting them into a chloroform vapor chamber. In a fume hood, place paper towels in a large glass or Pyrex dish. Lightly dampen the paper towels with water. Put the filters (colony side up) on the paper towels. In a small beaker, place a few Kimwipes or other absorbent paper material and soak with chloroform. Cover dish tightly with Saran wrap and leave at room temperature for 15 min.
4. Remove the filters from the chamber and place colony side up on a piece of

Whatman 3MM paper saturated with Colony Denaturing Solution. Use just enough of the solution to fully saturate the paper, since too much liquid will result in smearing of the signal. If done in a Petri dish this will require ~2.5ml of solution per filter. Leave in contact with the solution for 15 min at room temperature.

5. Perform the following incubations and washes at room temperature with gentle agitation. Filters may be placed in petri dishes (up to 5 per dish, given adequate agitation). Liquefy the 10% gelatin (supplied) by brief heating in a water bath or microwave oven. Block non-specific protein binding sites by immersing filters in TBST + 1% gelatin for 30 minutes (use about 5ml/filter). Most colonies will fall off the filters as soon as they are put in the blocking solution. TBST is 10mM Tris-HCl pH 8.0, 150mM NaCl, 0.05% Tween-20. It can be prepared as a 10X stock containing 0.02% sodium azide and stored at room temperature.
6. Wash with TBST (without gelatin) twice for 15 minutes each. Remove all visible colony debris by rubbing filter with a Kimwipe. Failure to remove colony debris results in a combination of high background and low signal and thus positives cannot be distinguished from background.
7. Incubate filters with primary antibody diluted in TBST for 30 minutes (5ml per filter; less can be used if filters are incubated together). If using the Positive Control Clone, dilute the Positive Control HSV•Tag Antibody 1:2000 to 0.1µg/ml). Many diluted antibodies can be used at least 4 times within a 2 week period, if they are preserved with 0.05% sodium azide and stored at 4°C.
8. Remove antibody solution and wash filters three times with 15-20ml TBST per filter for 10 minutes each.
9. Incubate filters for 30 minutes with Goat anti-Mouse IgG Alkaline Phosphatase conjugate diluted 1:10,000 in TBST. This diluted antibody conjugate can also be reused up to 4 times. Add sodium azide to 0.05% and store at 4°C.
10. Remove the antibody solution and wash filters three times with 15-20ml TBST for 10 minutes each.
11. After the last wash, place the filters on paper towels to absorb excess liquid, but do not allow them to dry out. Dilute the 20X stock of Alkaline Phosphatase Buffer to 1X with water and add 4µl/ml of each of the NBT and BCIP Color Development Substrates. Transfer the filters to Petri dishes and add enough color development solution to cover each filter completely (about 6ml). Strong signals generally develop within 1-10 minutes. Incubate until the background of the filters shows all of the colonies. This will make it easier to identify the original positive colony to rescreen. To stop color development, rinse the filters several times in water and allow to air dry. If colony density of the plate is high, it will be necessary to rescreen the colonies to obtain an isolated, pure colony. Restreak from the positive colony area to fresh plates. Rescreen the following day using the above protocol, and reuse the antibody solutions.