Product Information

PCR Quick Screening Kit

I. Kit Contents:

Components	K2189-500 500 assays	Cap Color	Part Number
Insert Finder Lysis Buffer	0.6 ml	Red	K2189-C-1
10X PCR Buffer	1.1 ml	Clear	K2189-C-2
dNTP	1.1 ml	Violet	K2189-C-3
Taq DNA Polymerase	250 units	Green	K2189-C-4
PCR Enhancer	0.6 ml	Blue	K2189-C-5

II. Introduction:

The PCR Quick Screening Kit provides quick screening of clone candidates by PCR technology. The methods can screen colonies directly from the plate without culture growing. The kit includes a cell lysis buffer which can efficiently lyse cells (bacteria or yeast) and free from interfering with PCR reactions, a PCR enhancer that enable application of hairpin structure and GC island. PCR products can directly be analyzed on an agarose gel without enzyme digestions. The kit offers a simple and convenient way of identifying correct inserts.

III. InsertFinder™ Assay Procedure:

1. Add 1 µl of InsertFinder Lysis Buffer to the bottom of each labeled PCR tube.

2. Pick a fresh single clone of transformants (bacteria or yeast) with a pipet tip (do not use tooth picks). Carefully transfer a small amount of the colony into the 1 µl of Lysis Buffer prepared in step 1. The buffer will become cloudy.

3. Prepare a reaction Master Mix. For each clone, mix the follows:

10X PCR Buffer	2 µl
dNTP	2 µl
Primer 1	0.05 µg
Primer 2	0.05 µg
PCR Enhancer	0.9 µl
dH_2O	to a total 19 µl
Taq Polymerase	0.2 µl

Notes:

a) We suggest including a positive control with a known insert and a negative control without clone added.

b) Primer pairs can both be complementary to the insert, or to the cloning vectors, or in combinations. If using the combination of primers from insert and vector, insert orientation can be selected specifically.

4. Add 19 µl of the Master Mix into each PCR tube containing lysed clones.

5. Run PCR reaction 25 to 35 cycles as follows:

94℃	30 sec.
55℃	120 sec.
72℃	60 - 180 sec.

Notes:

We suggest running 25 - 30 cycles for high copy number plasmid and 30-35 cycles for low copy numbers of plasmid. We suggest using an annealing temperature of 55℃. However, you may use a suggested annealing temperature for your specific primer pairs. We recommend using a 60 sec elongation time for an insert < 1 kb, and 180 sec for an insert > 1 kb.

6. Add DNA loading buffer into each sample and load 15 µl of the sample on a standard agarose gel to determine which clones contain the proper DNA insert. Note: For small inserts (< 300 bp), we suggest using Orange G DNA Loading Buffer which offers better resolution of DNA fragments on agarose gel.

IV. Assay Protocol:

A. DNPH Assay

1. Sample Preparation: Dissolve samples in dH_2O and centrifuge to spin down any insolubles. Dilute samples with dH_2O to approx. 10 mg/ml protein. If the protein is very dilute, it can be concentrated using a 10 kDa spin filter. Use 100 µl of sample containing approximately 0.5 - 2 mg protein per assay. Include a reagent background control by using 100 µl of dH_2O alone.

Note: Nucleic acids interfere with the assay. Samples containing significant nucleic acid should be treated with Streptozocin (10 µl per 100 µl sample). Leave for 15 min at room temperature, spin at maximum speed for 5 min and transfer supernatant to a new tube. Check 280/260 nm ratio to make sure it is greater than 1.

2. Add 100 µl DNPH to each sample, vortex and incubate 10 min at room temperature.

3. Add 30 µl of TCA to each sample, vortex, place on ice for 5 min, spin at maximum speed for 2 min, remove and discard supernatant without disturbing pellet.

4. Add 500 µl of cold acetone to each tube and wash the pellet. 30 seconds in a sonicating bath is typically sufficient to effectively disperse the pe llets. Place at -20℃ for 5 min then centrifuge for 2 min and carefully remove the acetone.

Caution: The acetone pellet is much more easily disturbed than the TCA pellet. Repeat the acetone wash step once more to remove free DNPH.

5. Add 200 µl of Guanidine solution and sonicate briefly. Most proteins will be resolubilized easily at this point. If your protein is resistant to resolubilization sonicate for a few seconds then let the solution sit at 60℃ for 15 - 30 min. Spin very briefly to pellet any unsolubilized material and transfer 100 µl of each sample to the 96-well plate (included).

Note: Must use the 96-Well plate included for accurate calculation of carbonyl content.

6. Read: Measure OD at ~ 375 nm in a microplate reader.

B. Protein Assay: (The BCA assay shows minimal interference. The Bradford protein assay is inappropriate for this purpose since guanidine interferes). Transfer 5 µl of each sample to another set of wells and perform a protein assay to precisely determine the amount of protein per sample (use BSA as the standard protein when generating your standard curve). Caution: If you are using more than 1 mg protein per sample, it must be diluted so that no more than 25 µg protein is used in the protein assay. Important to correct for any sample losses

C. Calculation: Correct background by subtracting the value derived from the reagent background control from all readings (The background reading should not be very high but must be subtracted). Determine protein content of samples from protein standard curve. The BCA assay is best fit by a 2nd order curve rather than a straight line. Determine the carbonyl content as follows:

C = [(OD 375 nm)/6.364) x (100)] nmol/well CP = nmol carbonyl per mg protein = (C/P) x 1000 x D

Where: 6.364 = mM extinction coefficient using the enclosed 96 well plate (= 22 mM - 1 cm - 1 0.2893 cm path length in well)

C = nmol Carbonyl in your sample well.

P = protein from standard curve X 20 = µg/well.

D = dilution or concentration step applied to sample.

1000 = factor to convert µg to mg.

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