Tech Tips

Xenographic Retention Chromatography (XRC)

Sample Incubation Optimization

Overview

This is a convenient procedure for conducting an experiment to determine the effect of varying sample incubation time with the Paramagnetic Particles on tumor cell recovery. Use this procedure as an example to optimize the incubation time for different sample types or different capture antibodies coupled with the Paramagnetic Particles.

Note

1) This procedure compares incubation times of specimen (i.e., whole blood, buffy coat, cell culture, etc.) spiked with a known count of preserved MCF-7 cancer cells.
2) The specimens are incubated with EpCAM Paramagnetic Particles at 30, 60, and 120 minutes.
3) After incubation, the specimens are assayed with one of the EpiSep™ Cytokeratin reagent kits.
4) Use this procedure as an example to test other sample types or other antibodies coupled with the Paramagnetic Particles.
5) You may choose to vary the incubation time based on your own results.

Materials & Reagents Required

- EpiSep™ Cytokeratin reagent kit.
- Preserved MCF-7 epithelial cells with known cell count.
- A specimen with adequate volume to be divided into 9 aliquots of 0.5 – 1mL each.
- 1.5 – 2.0mL microvial tubes, snap-cap indented top tubes are preferred to minimize sample loss in the cap.

Sample Preparation

1) Resuspend preserved MCF-7 cells by placing on Microvial Rotator for approximately 5 minutes and immediately prior to spiking the sample.
2) Spike the sample with a known concentration of preserved MCF-7 epithelial cells.
3) Invert the sample tube several times to ensure proper mixing.
4) Divide the sample into 9 equal aliquots by transferring 0.5 - 1mL of sample into nine 1.5 - 2.0ml microvial tubes.
5) Label the first 3 microvial (1 – 3) with the specimen ID and “120”.
6) Label the second 3 microvial (4 – 6)with the specimen ID and “60”.
7) Label the last 3 microvial (7 – 9) with the specimen ID and “30”.

Sample Incubation

1) Resuspend EpCAM MagParticles by vortexing briefly.
2) Resuspend the first 3 microvial (1 – 3), labeled “120” by placing on Microvial Rotator for approximately 5 minutes and immediately prior to the addition of the EpCAM MagParticles.
3) Add 50µL of EpCAM MagParticles to each of the first 3 microvial (1 – 3).
4) Begin the 120 minutes incubation by placing the three microvial on the Microvial Rotator. Note the time as zero minutes.
5) After 60 minutes have elapsed, prepare the second 3 microvial (4 – 6) labeled “60” in the same manner as vials 1 – 3, by resuspending the sample and the EpCAM MagParticles.
6) Add 50µL of EpCAM MagParticles to each of the second 3 microvial (4 - 6).
7) Begin the 60-minute incubation for microvial 4 – 6 by placing on the Microvial Rotator. Note the time as 60 minutes.
8) After 90 minutes have elapsed, from time zero, prepare the last 3 microvial (7 – 9) by resuspending the sample and the EpCAM Paramagnetic Particles.
9) Add 50µL of EpCAM MagParticles to each of the last 3 microvial (7 – 9).
10) Begin the 30-minute incubation for microvial 7 – 9 by placing on the Microvial Rotator. Note the time as 90 minutes.
11) Follow instructions in one of the EpiSep™ Cytokeratin directional inserts for preparing the EpiSep™ Slides.
12) After 30 more minutes have elapsed, now 120 minutes from time zero, transfer all the samples (microvial 1 – 9) completely to the EpiSep™ Slides using a transfer pipette.
13) Proceed with completing the assay following the instructions in one of the EpiSep™ Cytokeratin directional inserts.

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Viewing Results

1) After the assay is complete, view and count recovered tumor cells and/or preserved MCF-7 epithelial cells using an inverted fluorescence or confocal microscope.
2) Calculate the average for each set of three tubes, 1 – 3, 4 – 6, and 7 – 9.
3) Compare the counts of recovered cells for the three sets with the known count for the original sample.
4) Use these counts to determine the optimal incubation time for cell recovery.

Technical Assistance

For technical assistance, contact the WaveSense, Inc. Technical Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

Customer Service

To place an order, contact the WaveSense, Inc. Customer Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

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