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ZNF384 Break Apart FISH Probe

Color(s): Green/Orange

REF	ZNF384BA-10-GROR	**Quantity:** 10 Tests, 20µl
REF	ZNF384BA-20-GROR	**Quantity:** 20 Tests, 40µl
REF	ZNF384BA-CS-GROR	**Quantity:** See Package Label

CE

For *in vitro* use only
CE marked in certain countries
RUO in US and other countries

Storage, Handling, Shelf Life, and Disposal: Store product at -20°C; avoid light; expiration noted on product label. If packaging is damaged, please notify your local distributor. Gloves and other protective equipment should always be worn when handling probe. Empire Genomics' probes are light sensitive and should not be exposed to excessive light. Handle probes in a dark place to avoid bleaching. Dispose as per local regulations.

Composition:
- Concentrated labeled FISH Probe: Minimum 20ng/µl
- Hybridization Buffer (Contains small concentration of formamide)

Intended Use:

Empire Genomics' ZNF384 Break Apart FISH Probe is a fluorescence in situ hybridization (FISH) test designed to detect rearrangements involving the ZNF384 gene in the 12p13.31 chromosomal region. This is a qualitative, non-automated test designed to be used as an adjunct to other clinical tests and is not to be used as the sole method for diagnosis.

Specimen Types:
- Peripheral Blood
- FFPE Tissue
- Bone Marrow

Warning and Precautions: Product does not contain any human or animal components. See safety data sheet for more detailed safety and handling information. Contains formamide in low concentration. Do not use expired probe. Do not reuse probe. Avoid cross-contamination. Read instructions for use in full before use.

- H360d May damage the unborn child.
- P309+P310 IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

- P201 Obtain special instructions before use.
- H315+H320 Causes skin and eye irritation.
- P262 Do not get in eyes, on skin, or on clothing.

- P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P501 Dispose of contents/container to local/regional/national/international regulations.
- P280 Wear protective gloves/protective clothing/eye protection/face protection.

Performance Characteristics:

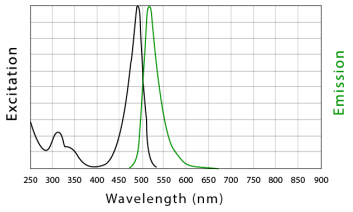
This FISH probe was tested on normal blood spreads. As a part of quality control the probe was subjected to analysis of signal strength and specificity. Please see included Certificate of Analysis for more details. Accuracy has been determined to be 100%. This probe is designed to only hybridize to the regions specified on the below ideograms.

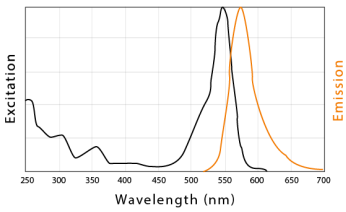
Limitations:
- This product is for *in vitro* use only.
- Probe performance is dependent on sample preparation, sample quality, and proper product storage.
- This product is only for use by trained laboratory professionals.

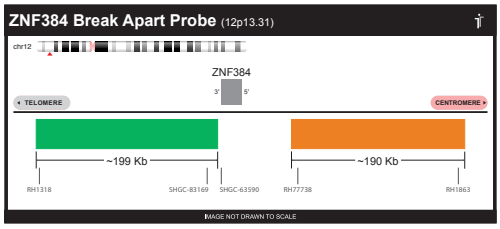
Installation and Operation: All equipment used in the FISH experiment must be properly calibrated. Filters and light source used to detect fluorescent signals must be routinely replaced to ensure optimal probe performance. Temperature and humidity control are important for the probe to operate properly, ensure all thermometers and hydrometers are calibrated. Probe should be evaluated on normal samples to ensure proper hybridization.

Principle of Method: Fluorescence *in situ* hybridization (FISH) is a cytogenetic technique that is used to detect genomic aberrations. FISH probes are commonly used to detect deletions, amplifications, and rearrangements of a genomic target.

Color	Absorbance Maximum	Emission Maximum
Orange-dUTP	548 nm	573 nm
Green-dUTP	491 nm	515 nm







Reagents Not Provided

For Non-Tissue Slide Preparation:
- 70% ethanol
- 100% methanol
- Acetic acid

For FFPE Pretreatment
- 0.01N HCL
- Xylene
- Ethanol (70%, 85%, 100%)
- 10mM Citric acid (BDH, 4136-500G)
- 160mg solid Pepsin (Sigma, # P7012-1G)
- 0.3% Igepal, CA-630 Sigma (or NP40)/0.4XSSC
- 0.1% Igepal CA-630, Sigma (or NP40)/2XSSC

Required Equipment:
- Fluorescent Microscope with appropriate filter set.

For Automated Hybridization
- Wash Solution 1 (WS1) – 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC
- Absorbent material
- dH_2O
- Wash Solution 2 (WS2) – 0.1% Igepal (Sigma CA-630) or NP-40 / 2 x SSC
- DAPI with Antifade

For Manual Hybridization
- Denaturation Buffer – 70% Formamide, 2 x SSC, pH 7.0-8.0
- 70%, 85%, 100% Ethanol
- Wash Solution 1 (WS1) – 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC
- Wash Solution 2 (WS2) – 0.1% Igepal (Sigma CA-630) or NP-40 / 2 x SSC
- DAPI with Antifade

Slide Preparation Protocol for Fixed Cell Pellet

Notes:
- Ideal environmental conditions for slide making are between 45-55% humidity and 24°C (72-75°F) with minimal drafts. A hydrometer/thermometer should be positioned near the slide making bench to monitor environmental conditions. If conditions are not ideal, adjustments should be attempted prior to slide making.
- Specimen quality is important and difficult to adjust once fixative is added. It is important to follow standard Cytogenetics* protocols using reagents that have been tested prior to use.
- Optional: Clean slides by placing them in a coplin jar with 70% ethanol for 5 minutes followed by wiping in one direction several times with a tissue. After this, place the slide in a coplin jar with fresh 3:1 methanol:g acetic acid fixative. Slides may be used directly, or dried and stored in a freezer (-20°C)
- Make slides on only one patient sample at a time to avoid cross-contamination between specimens.

Step 1 - Slide Making

1. Harvest the sample using standard Cytogenetics* protocols.
2. Change fixative (Carnoy's 3:1 methanol:g acetic acid) in sample tube until supernatant is colorless. Re-fix sample in fresh fixative just prior to slide making.
3. Set slide warmer to ~45°C
4. Aspirate supernatant ~5ml above cell pellet and re-suspend cells using a glass Pasteur pipette.
5. Add enough fresh cold fixative to produce a slightly milky suspension.
6. Remove one slide from fixative coplin jar, drain on paper towel. Alternatively remove one cold slide from freezer.
7. Hold slide slightly vertical and drop 3 drops of suspension at top, middle, and bottom of slide.
8. Gently rotate slide, tipping slightly after ~15 seconds to drain excess suspension onto a tissue.
9. Keep slide horizontal until a grainy appearance is observed and the edges of the slide begin to dry. Time will vary depending on atmospheric humidity.
10. Wipe back of slide with a tissue, and air-dry slides.
11. Place slide directly on slide warmer to keep dry.
12. Label slide with the appropriate patient slide label. Never leave an unlabeled slide on slide warmer. To label use HB pencil or permanent alcohol-resistant marker. Label slide with at least two unique patient identifiers, date and initial.
13. Assess slide under phase contrast microscope (10x objective)

Step 2 - Assessing Slide Quality

Ideally the concentration should have ~50 interphase cells/field; interphase cells should be large, gray, and flat. Metaphases should be well spread with minimum crossovers, intact (not overspread), no cytoplasm, and dark gray in color. If they are not, consider the following variables.

- **Concentration Too Thick:** Cells will be under spread and probably be in cytoplasm (metaphases will appear in 3D with a distinct halo surrounding them). Add additional fix accordingly.
- **Concentration Too Thin:** Metaphase may not be intact, more probe required for larger surface area. Re-centrifuge tube (1200rpm for 10 minutes) and remove excess fix accordingly.
- **Dried Too Fast**: interphase cells appear refractile, small, and black. Metaphase cells are under spread, in cytoplasm, and remained black. If humidity level is too low (below 40-45%), create a more humid environment by doing one of the following: add a room humidifier, make slides over a beaker of steam, make slides over a sink with running hot water, or place wet tissues on slide warmer and place slide briefly (~5 seconds) before placing directly on slide warmer.
- **Dried Too Slowly:** Metaphases are light grey and may be over spread or not intact. Both scenarios might produce low-quality hybridization. Decrease time in steps 9-10 prior to placing slide on slide warmer. If humidity level is too high (above 55%) a room dehumidifier may assist in lowering humidity level.
- **Cytoplasm Around Metaphase:** If cytoplasm around metaphases continues to be an issue after making adjustments in steps above, add 1-2 drops of fixative to slide after steps 1-9 above.
- **Correct Drying**: Interphase cells are flat, plump, and pale; metaphase cells are dark gray, well spread with few cross-overs, and intact with no cytoplasm.

Step 3 - Slide Aging/Storage

1. Store dried slides in a desiccator for at least 24 hours at room temperature to age sufficiently before the FISH step. If results are required quickly, leave the slide on the slide warmer for at least 15 minutes prior to FISH.
2. If slide will not be hybridized within 24-48 hours it may be stored in a sealed container in a freezer (-20°C) for up to two weeks.
3. Fixed cell suspensions should be stored in cryovials in the freezer (-20°C).

***References**

1. Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual Third Edition. Chapter 3 - Peripheral Blood Cytogenetic Methods (M.G. Brown, H.J. Lawce). Lippincott-Raven Philadelphia 1991
2. Dunn B, Mouchrani P, Keagle M. The Cytogenetic Symposia - AGT. Second edition.

Slide Processing for Paraffin-Embedded Tissue Samples

This procedure describes the steps involved in slide processing of paraffin embedded tissue. The pretreatment process de-paraffinizes and pre-treats the sample prior to denaturation and hybridization with appropriate probes. Following pretreatment, the slides and appropriate probes are denatured. Proceed to appropriate hybridization instructions after processing is complete.

Deparaffinization and Pretreatment

- Preheat 10mM citric acid to 90-95°C
- Preheat 40mL 0.1N HCL to 37°C
- Heat oven to 90°C
- Prepare 1mL of 160 mg/mL pepsin in H_2O

1. Age slides at 90°C for 25 minutes in oven.
2. Immerse slides in xylene for 10 minutes. Repeat one time with fresh xylene.
3. Immerse slides in 100% Ethanol for 5 minutes. Repeat once.
4. Air dry on slide warmer.
5. Place slide in 90-95°C citric acid pretreatment buffer (pH ~6.8) for 30 minutes.

Digestion

1. Add 1 mL of 160 mg/mL Pepsin solution to 40mL 0.1N HCL and mix well.
2. Place slide in Pepsin solution for 20-30 minutes.
3. Wash slides in 2x SSC for 5 minutes
4. Immerse slides in 70% ethanol for 30 seconds. Air dry slide or place on slide warmer to dry.
5. View digestion under microscope. If adequate digestion is not acheived, repeat steps 2-4, but change digestion time to 10 minute increments and analyze slides after each digestion.
6. When adequate digestion is acheived, dehydrate slides through 70%, 85%, and 100% Ethanol respectively for 2 minutes each. Proceed to hybridization protocol.

Automated Hybridization Instructions

These instructions are for hybridizations that will be carried out using an automated Hybrite/Thermobrite. If a Hybrite/Thermobrite will not be used, then follow "Manual Hybridization Instructions".

Notes

- Protocol can be used with all FISH probes – controls, gene specifics, custom FISH probes.
- Solutions can be made prior to the procedure.
- Further optimization of the protocol may be required.

1. Turn on Hybrite/Thermobrite.
2. Set Program guide (see "Program Guide Options").
3. Presoak absorbent material in dH_2O.
4. Add 2µL of probe and 8µL of hybridization buffer.
5. Apply a clean 22 x 22 coverslip
6. Apply rubber cement on edges of coverslip to seal.
7. Place in Hybrite/Thermobrite and close lid.
8. Start the program.
9. Let run for at least 16 hours.
10. Pre-warm WS1 to 73°C.
11. Remove coverslip, and place slide in agitating WS1 for ~10 seconds.
12. Let slide stand for exactly 2 minutes.
13. Transfer slide to WS2 at room temperature. Agitate for ~10-15 seconds and let the slides sit for 2 minutes
14. Let slide dry in the dark.
15. Apply 10µL of DAPI with Antifade and cover with a 22x50 coverslip.
16. Wait 15-30 minutes, then visualize under microscope with appropriate filter.

Program Guide Options

For Peripheral Blood Preparations

- Denature at 72-73°C for 2 minutes.
- Hybridize at 37°C for at least 16 hours.

For Paraffin Embedded Tissue After Pretreatment

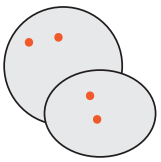
- Denature at 75°C for 7 minutes.
- Hybridize at 37°C for at least 16 hours
  May Require Troubleshooting

Manual Hybridization Instructions

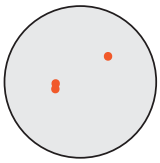
These instructions do not use a Hybrite/Thermobrite. Following these instructions will allow the probe to properly hybridize to a sample without an automated system. If an automated system like a Hybrite/Thermobrite will be used, follow "Automated Hybridization Instructions" instead.

1. Warm specimen slide to room temperature.
2. Place slides in 70% Ethanol at room temperature for 2 minutes.
3. Place slides in 85% Ethanol at room temperature for 2 minutes.
4. Place slides in 100% Ethanol at room temperature for 2 minutes.
5. Gently dry back of slide and place on slide warmer at 45°C until Ethanol evaporates.
6. Prepare probe mixture by mixing 2µL of probe with 8µL of buffer.
7. Pipette probe 10µL mixture onto slide.
8. Apply clean 22 mm^2 coverslip to slide.
9. Seal edges of coverslip with rubber cement.
10. Denature slide on a hotplate according to "Program Guide Options" above. Ensure your slide is shielded from any light exposure during this process.
11. Place slide in a pre-warmed 37°C humidified chamber, and place the chamber in a 37°C incubator.
12. Incubate at 37°C for 16 hours.
13. Pre-warm WS1 (0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC) to 73°C.
14. Remove coverslip. Place in WS1, agitating for approximately 10 seconds then let stand for exactly 2 minutes.
15. Transfer slide to WS2 at room temperature. Agitate for ~10-15 seconds and let the slides sit for 2 minutes.
16. Let dry in dark.
17. Apply 10 µl DAPI with Antifade and cover with a 22x50 coverslip.
18. Wait 15-30 minutes then visualize under microscope using the appropriate filter sets

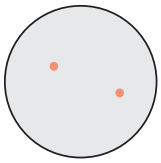
Assessing FISH Quality:



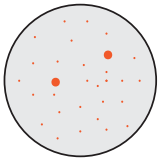
Overlapping nuclei: Do not read these cells.



Dual signal on one chromosome: Count dual signal as a single signal.



Weak Signal: See troubleshooting guide. Can still read if signal is clear.



High background signals: See troubleshooting guide, can still read cell if not too severe.

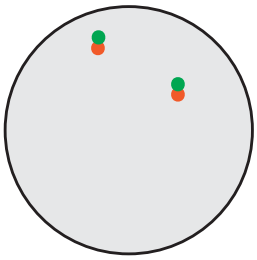


Figure 1

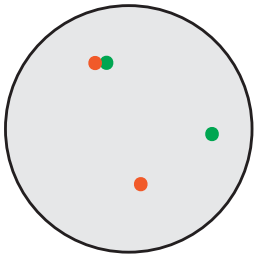


Figure 2

Figure 1: Normal signal pattern displaying 2 green/orange fusion signals.

Figure 2: Normal signal pattern displaying 1 green/orange fusion signal, 1 green signal and 1 orange signal indicating a rearrangement involving ZNF384.

Troubleshooting FAQs

Distorted Chromosome Morphology

Possible Reason: Slides dried too quickly

Potential solutions:

- Increase humidity when dropping slides.
- Increase time after dropping slide before placing it on slide warmer.
- Ensure slide warmer is ~45°C. Monitor temperature with surface thermometer.
- Refix cell suspension in freshly prepared 3:1 methanol:g acetic acid fixative.

Possible Reason: Slides aged/stored improperly

Potential solutions:

- Age slides at least 24 hours at room temperature prior to FISH.
- Do not bake slides.
- If FISH needs to be performed the same day as slide preparation, incubate slide for at least 1 hour in coplin jar containing 2 x SSC at 37°C, then dehydrate slide for 1 minute in 70%, 85%, and 100% ethanol respectively prior to denaturation step.

High Background/Low Specificity

Possible Reason: Glass slides are not clean

Potential solutions:

- Soak slides in 70% ethanol for 5 minutes, then wipe 2-3 times with a tissue.

Possible Reason: Poor specimen quality with cellular debris

Potential solutions:

- Wash cell pellet with fresh fixative 2-3 times, then repeat slide making. Ensure that the cell suspension is not too thick.

Possible Reason: Cytoplasm around chromosomes

Potential solutions:

- Slides dried too quickly. See above

Possible Reason: Wash solutions incorrect

Potential solutions:

- Ensure WS1 and WS2 are prepared and stored properly.
- Check pH (7.0) and temperature (73°C) of wash solutions are correct. Place thermometer directly into WS1.
- Ensure wash solutions are not expired or overused. Discard after 10 slides.
- Ensure only 4 slides are washed at once to maintain proper temperature of WS1.
- Ensure time in wash solutions is appropriate.
- Increase time in WS1 to 3 minutes.

Possible Reason: Broadband past microscope filters

Potential solutions:

- Use filters with narrow bandwidths specific to fluorochromes used.

Possible Reason: Inadequate hybridization conditions

Potential solutions:

- Use tightly sealed chambers with appropriate humidity control.

Weak or No Signal

Possible Reason: Slide not adequately denatured

Potential solutions:

- Ensure denaturation temperature is correct by capturing a temperature of the hotplate or automated hybridizer using a temperature gun or other device.
- Troubleshoot by increasing denaturation temperature.
- Ensure time in denaturation is correct following the “Program Guide Options” above. Increase or decrease time if necessary.

Possible Reason: Probe not adequately prepared

Potential solutions:

- Completely thaw probe and hyb buffer (15 minutes at room temperature in a dark environment). Add hyb buffer to probe. Vortex and centrifuge briefly.

Possible Reason: Probe exposed to light or stored incorrectly

Potential solutions:

- Perform FISH in a dimly lit room. Store probes at -20°C. Avoid excessive freeze/thaw cycles.

Possible Reason: Microscope specifications inadequate

Potential solutions:

- Ensure UV light source is adequate for viewing FISH signals. If unsure, contact microscope manufacturer.
- Ensure UV light source is centered.
- Ensure proper filters are installed for fluorochromes used. If unsure, contact microscope manufacturer.
- Ensure filters are not damaged.
- Ensure wash solutions were prepared properly.