

TRU BLOCK[®]

*Heterophilic Antibodies
Mouse IgG
IVD Manufacture Data
Optimizing Evaluations*



Meridian
Life Science, Inc.

www.MeridianLifeScience.com

HA INTERFERENCE



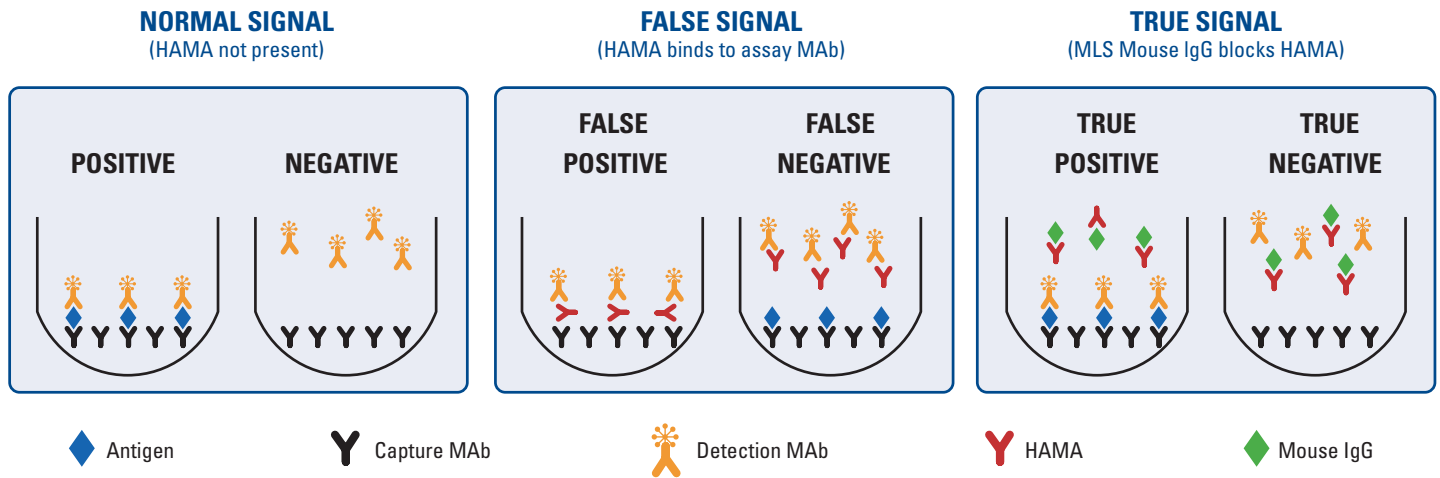
Immunoassays used for human in vitro diagnostics (IVD) often use animal-sourced antibodies to recognize specific disease markers.

A small percentage of individuals, however, may have antibodies in their blood that could react with the animal antibodies in the diagnostic assay and thereby interfere with detection of the disease markers causing false positive or false negative results. Such an interfering antibody is termed a heterophilic antibody (HA).

Due to the wide use of mouse monoclonal antibodies in diagnostic applications, the most well-known HA interference is a result of HAMA (human anti-mouse antibodies).

Rheumatoid factor (RF), an autoantibody that reacts with the patient's own immunoglobulin (Ig), may also cross-react with animal Ig resulting in RF interference, which is similar to HA/HAMA interference.

Much like HAMA, HA to other animals such as goat (HAGA), sheep (HASA), and rabbit (HARA) may also cause false results, especially when antibodies originating from such animals are used in immunoassays.



The blocking mechanism of typical HAMA Blocker mouse IgG is shown above.

Heterophilic antibodies (HA) to other animals may also cause false results. MLS has available the following animal IgG's for HA blocking:

Sheep (HASA) | Goat (HAGA) | Rabbit (HARA)



MOUSE IgG



The frequency of HA interferences is low. The false results have a significant negative impact on the quality and competitiveness of diagnostic assays as well as on the lives of those individuals who have been falsely diagnosed.

IVD manufacturers often use mouse serum or mouse Immunoglobulin G (IgG) to block HAMA interference in their assay. Limitations to this method include:

- Mouse IgG blocks HAMA but not other types of HA interference
- Mouse IgG does not block RF interference

Mouse IgG blocks HAMA interference passively by being competitively bound to a HAMA molecule at the same affinity as the assay antibodies. High concentrations of mouse IgG (up to 10x the concentration of the assay antibodies) are required to show sufficient blocking. This presents a challenge in miniaturized immunoassays where reduction in the amount of assay components is desired.

Mouse IgG alone also presents a significant limitation due to the need for broader coverage against various HA/RF interferences in IVD immunoassays.

Meridian Life Science[®], Inc. has developed an active HAMA blocker called TRU Block[®].

DIFFERENCES BETWEEN TRU BLOCK AND MOUSE IgG

TRU BLOCK	MOUSE IgG
Active Blocker	Passive Blocker
Uses a proprietary active mechanism to bind HAMA	Passively binds by acting as an antigen in a reaction with HAMA
Works well on HAMA as well as other types of HA	Effective on HAMA but not on other types of HA
Much lower concentration is needed	Higher concentration is needed
5-20 times more effective than Mouse IgG for blocking HAMA	

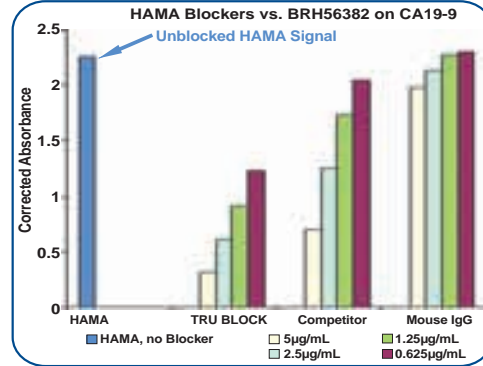


HAMA / RF – TRU BLOCK PERFORMANCE

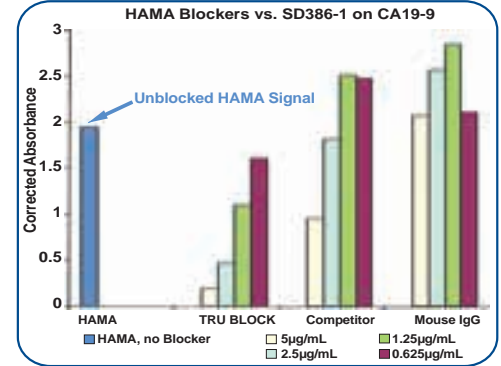


HAMA ASSAY METHOD

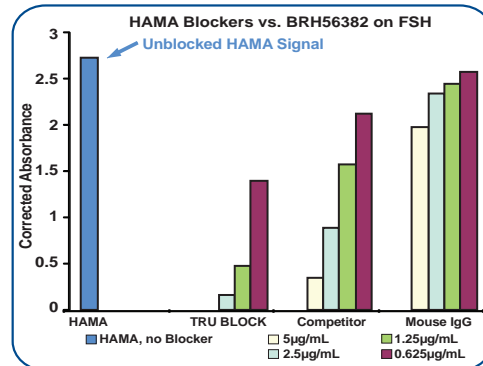
- Two commercial sandwich immunoassays were used: CA 19-9 and FSH.
- Buffer lacking mouse Ig was used in place of kit buffer which contained mouse IgG as blocker.
- Six different HAMA-reactive sera were tested.
- Three different HAMA blockers were added in sample diluent buffer separately at four different concentrations.
- HAMA activity was measured initially in the absence of each blocker to determine 100% signal.
- HAMA activity was then tested in the presence of blocker to measure suppression of signal.
- Greater suppression of HAMA signal implies potency of blocker.



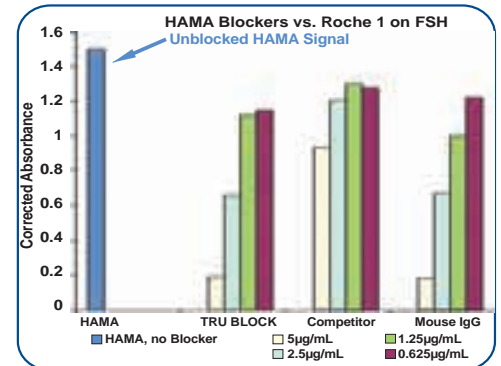
In the CA19-9 Assay, TRU Block outperforms Competitor's active blocker on BRH56382 HAMA sample.



In the CA19-9 Assay, TRU Block outperforms Competitor's active blocker on SD386-1 HAMA sample.



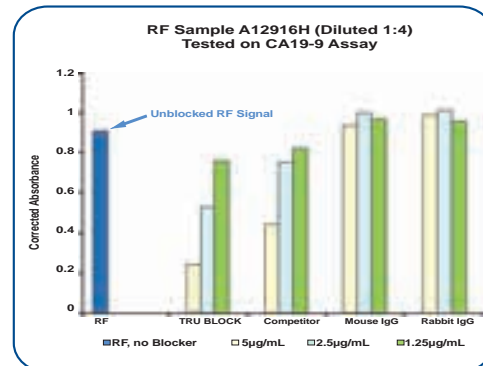
In the FSH Assay, TRU Block outperforms Competitor's active blocker on BRH56382 HAMA sample.



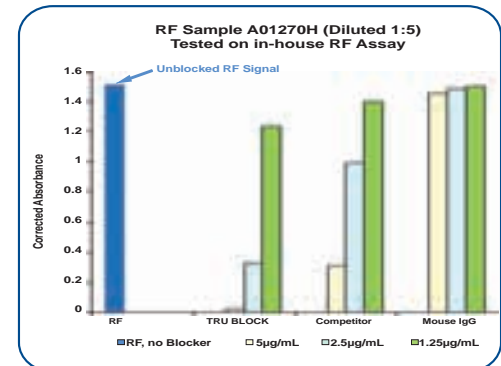
In the FSH Assay, TRU Block outperforms Competitor's active blocker on Roche Type I HAMA sample.

RF ASSAY METHOD

- The previous mentioned CA19-9 assay was used to test the Rheumatoid Factor (RF) serum A12916H. An in-house assay was used to test the A01270H RF sample.
- Blockers were added in sample diluent buffer.
- Only active blockers are effective in the above RF samples tested.
- Both RF serums are available from Meridian Life Science, Inc.



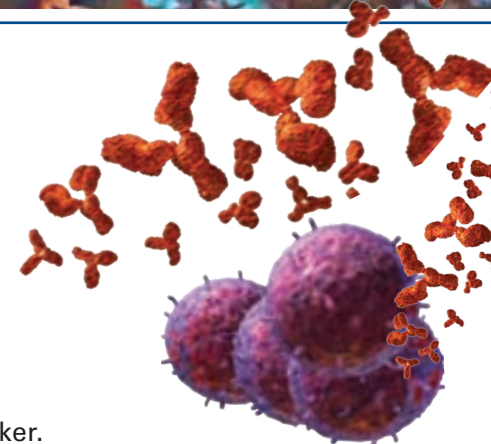
Based on CA19-9 Assay, TRU Block outperforms the competitor's active blocker on A12916H RF sample.



Based on the in-house assay, TRU Block outperforms the competitor's active blocker on A01270H RF sample.

TRU BLOCK OUTPERFORMS COMPETITORS ON HAMA AND RF SAMPLES.

IVD MANUFACTURER RESULTS



SAMPLING OF IVD MANUFACTURER RESULTS*

IVD 1: Cost Savings

- TRU Block performed ~30% better than existing active Blocker.
- TRU Block also costs ~30% less than existing active Blocker.
- Estimated savings >\$1 Million to have TRU Block fully replace existing active blocker.

IVD 2: Performance Gains

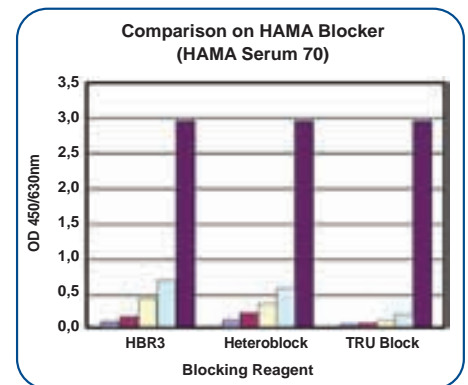
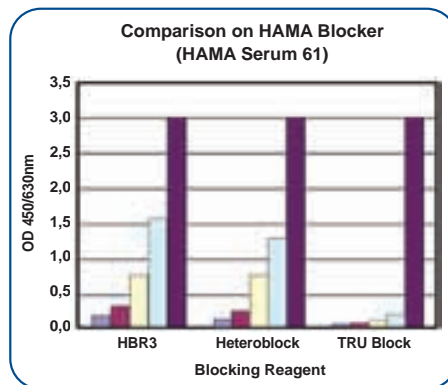
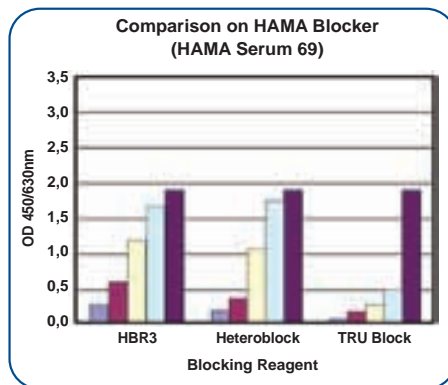
- TRU Block, used at 1/3 the concentration of the competitor, performed equivalent to the competitor.
- 60% performance/cost advantage.

IVD 3: Half and Half Approach

- Client was able to eliminate additional interference samples with TRU Block.
- Client decided to pursue the “Half & Half approach”: Mix TRU Block with existing blocker 50% / 50%.
- Reduced risk since existing blocker is not removed.
- Increased performance as new formulation will eliminate more interference than with the previous formulation.

IVD 4: Assay Information

- TRU Block performed significantly better than HBR-3 and Heteroblock.
- Obvious performance advantages allowed client to justify validation with ease.
- Client decided to validate TRU Block in all of their immunoassays.
 - **Blockers were tested in One-step MAb/MAb Sandwich ELISA:**
 1. Assay buffers with or without blockers were dispensed directly into the wells.
 2. Samples (HAMA positive) and a Biotinylated detection antibody were added to the wells.
 3. The mix was then incubated at room temperature for 2 hours.
 4. After washing, Streptavidin-HRP was added and incubated for 30 minutes.

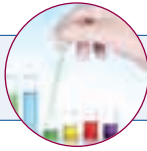


■ 250µg/mL ■ 125µg/mL ■ 62.5µg/mL ■ 31.25µg/mL ■ no blocker

*All results are dependent on assay platforms.

TRU BLOCK OUTPERFORMS COMPETITORS IN CUSTOMER APPLICATIONS.

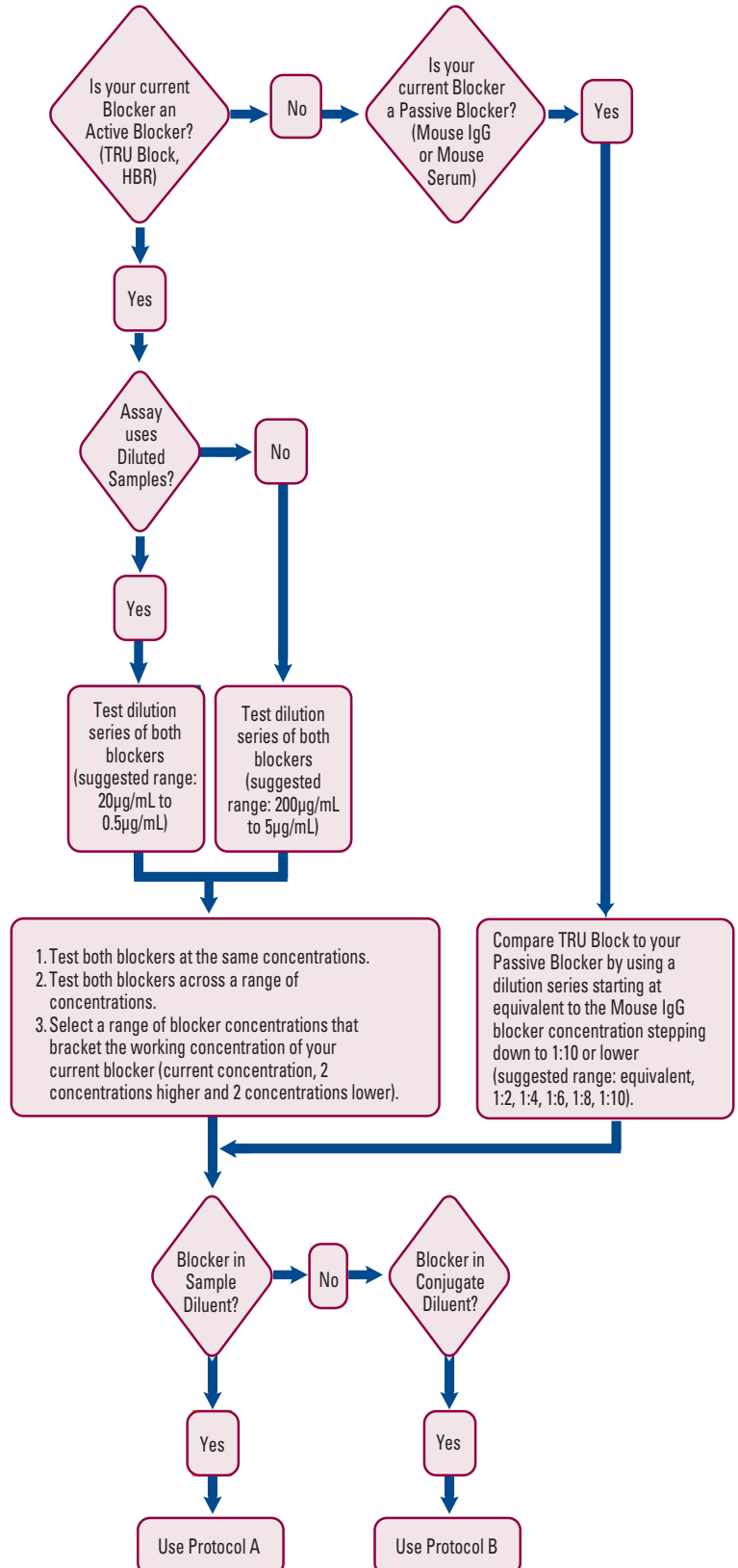
TRU BLOCK PROTOCOLS



IMPORTANT RECOMMENDATIONS FOR PLANNING YOUR COMPARISON OF TRU BLOCK TO YOUR CURRENT BLOCKING REAGENT

1. Use in any sandwich immunoassay that utilizes 2 mouse monoclonal antibodies after modification to remove HAMA blocking reagents that may be present in the kit buffers.
2. Perform a side-by-side evaluation of current blocker and TRU Block.
3. For optimum performance it is best to have the HAMA blocker in contact with the sample prior to the monoclonal antibodies. This is best accomplished by adding the HAMA blocker to a sample diluent. If your assay does not use a sample diluent, the HAMA blocker may be added to the conjugate diluent and then perform a 1-step assay (simultaneous incubation of sample and conjugate in the test wells). In lateral flow assays, the HAMA blocker may be added to the conjugate pad, to a sample diluent or pretreatment buffer, or applied to the membrane as a blocking stripe located before the test stripe.
4. To quantify % Blocking differences between blockers or blocker concentrations, the most sensitive assays will test the HAMA samples with unblocked HAMA activity in the linear range of the assay. For example, samples with very high unblocked HAMA/RF signals can be diluted to ensure the unblocked signal is within OD 1.000 - 2.000 (the linear range of the assay).
5. Remove your current blocker from any diluents/ buffers used in the assay before evaluation of TRU Block.
6. Select only Human Serum or Plasma samples for evaluation that contain known interference by HAMA, HA and RF. Non-human (plant or animal) samples, feces, urine or other non-blood samples do not contain HAMA and are not appropriate for evaluation of HAMA blockers. When testing purchased samples, it is important to understand if the interference in your assay is caused by HAMA, HA, or RF in the sample. Assay interference may also be caused by non-specific binding or some other type of interference. Only samples with known interference by HAMA, HA and RF should be used for evaluation of HAMA blockers.
7. Include Positive Controls (Unblocked HAMA Samples) and Negative Controls (Buffer only - No HAMA Sample) in each assay.

TRU BLOCK PROTOCOL SELECTION FLOW DIAGRAM



TRU BLOCK PROTOCOLS



PROTOCOL A

Pos control = Unblocked HAMA sample (no blocker in sample diluent)

Neg control = Buffer only (no HAMA sample)

1. Prepare conjugate diluent buffer without blocker.
2. Dilute blockers (TRU Block vs. current blocker) in sample diluent buffer across a range of working concentrations.
3. Dilute HAMA sample(s) with Sample Diluent containing blockers.
4. Dispense diluted HAMA sample(s) to test wells.
5. Incubate (according to assay protocol).
6. Wash test wells.
7. Dispense conjugate to test wells.
8. Incubate (according to assay protocol).
9. Wash test wells.
10. Add substrate to test wells.
11. Incubate (according to assay protocol).
12. Add Stop Solution.
13. Read OD values.
14. Calculate % Blocking.
15. Compare TRU Block performance vs. Current Blocker.

PROTOCOL B

Pos control = Unblocked HAMA sample (no blocker in conjugate diluent)

Neg control = Buffer only (no HAMA sample)

1. Prepare conjugate diluent buffer without blocker.
2. Dilute blockers (TRU Block vs. current blocker) in conjugate diluent buffer across a range of working concentrations.
3. Dispense HAMA sample(s) to test wells. Add conjugate diluted in conjugate diluents containing blockers.
4. Incubate samples + conjugate/blockers in test wells simultaneously (according to assay protocol).
5. Wash test wells.
6. Add substrate to test wells.
7. Incubate (according to assay protocol).
8. Add Stop Solution.
9. Read OD values.
10. Calculate % Blocking.
11. Compare TRU Block performance vs. Current Blocker.

CALCULATE RESULTS FOR BOTH PROTOCOLS

1. Calculate the % HAMA signal blocked: % Blocking = $[1 - (\text{Mean Blocked HAMA} - \text{Mean Negative Control}) / (\text{Mean Unblocked HAMA} - \text{Mean Negative Control})] \times 100$
 - Compare % Blocking at same blocker concentration.
 - Compare blocker concentrations that give same % Blocking.
2. Compare # of HAMA/RF samples resolved by each blocker.

Catalog #: A66800H

Product Name: TRU Block

Description: TRU Block is a complex blocker that contains active ingredients along with purified mouse IgG.

Source: Normal mouse serum plus proprietary ingredients

Format: Purified, Liquid

Purity: > 95% pure

Concentration: ~25mg/mL (OD280nm, E^{0.1%} = 1.4)

Buffer: 10mM Sodium phosphate, 150mM Sodium chloride, pH 7.2

Preservative: 0.05% Sodium azide

Applications: Active blocking of heterophilic antibody interference in immunoassays.

Storage: Short-term store at 2 - 8°C. Long term store at -20°C. Avoid multiple freeze and thaw cycles.

Warnings: This product contains sodium azide, which has been classified as Xn (Harmful) in European Directive 67/548/EEC in the concentration range of 0.1 – 1.0%. When disposing of this reagent through lead or copper plumbing, flush with copious volumes of water to prevent azide build-up in drains.

References: Sha, M., Hillman, P., Van Cleave, V., Larson, R. (2010) "Reducing Errors in Immunoassay," Genetic Engineering and Biotechnology News:30:15

ADDITIONAL PRODUCT INFORMATION

Sample Size: 100mg

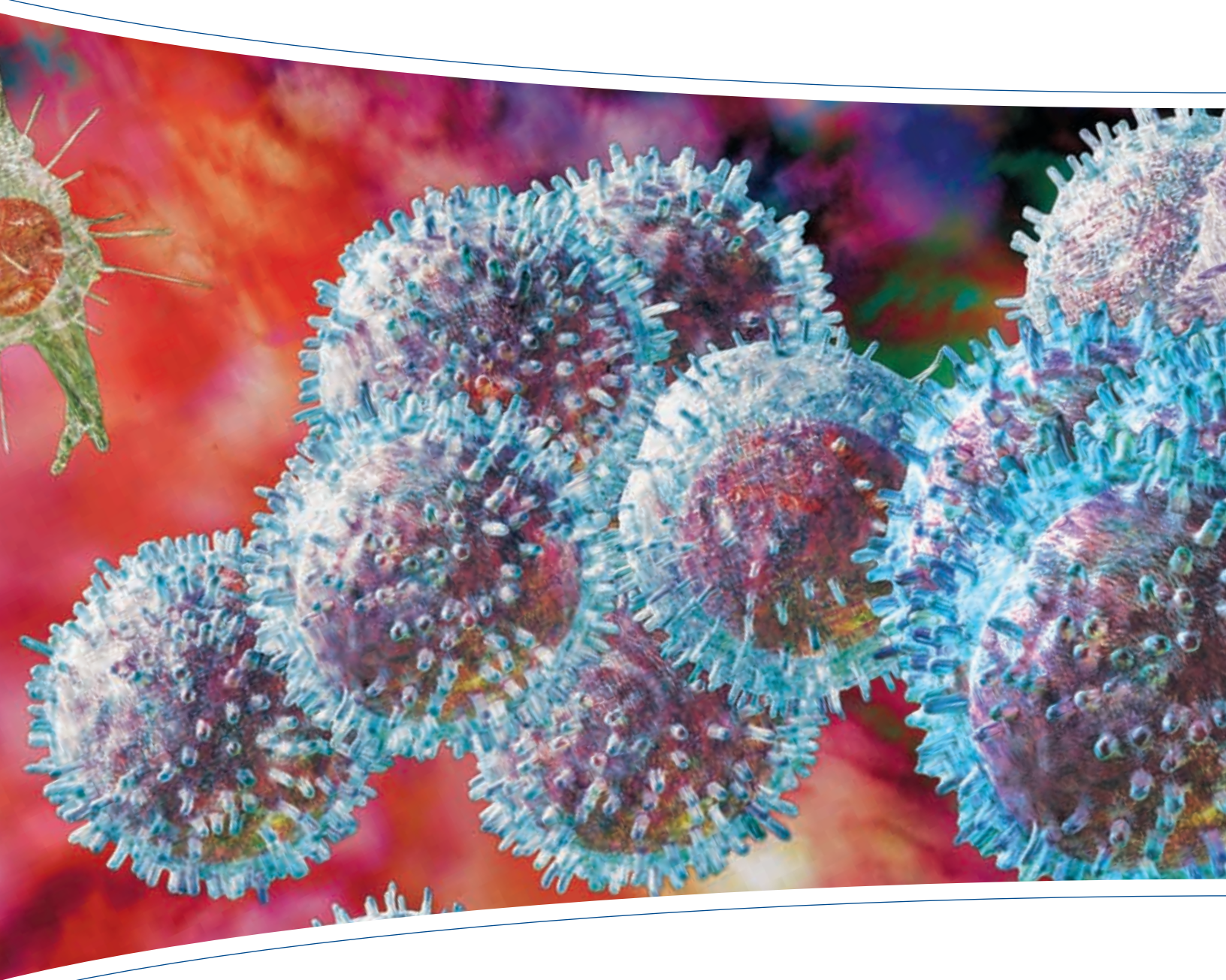
Package Sizes: 1g, 5g, 10g

Shelf Life: 5 years from Date of Manufacture @ -20°C

Shipping: Blue Ice.

Conditions: Dry Ice available upon request.

FOR RESEARCH AND FURTHER MANUFACTURE USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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