Human Immunodeficiency Virus (HIV)

OVERVIEW OF THE HIV INFECTION AND DIAGNOSTIC TESTING GUIDELINE

Human Immunodeficiency Virus (HIV) belongs to the genus *Lentivirus* within the *Retroviridae* family. It primarily targets CD4+ T lymphocytes. Left untreated, it can lead to acquired immunodeficiency syndrome (AIDS), a condition characterized by severe immune suppression and increased susceptibility to opportunistic infections and certain cancers.

According to the 2018 Quick Reference Guide from the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL), initial laboratory testing for HIV should be performed using an FDA-approved combo antigen/antibody immunoassay. This assay is designed to detect both HIV-1 and HIV-2 antibodies, as well as the HIV-1 p24 antigen, which enables the identification of both established HIV-1/HIV-2 infections and acute HIV-1 infection.

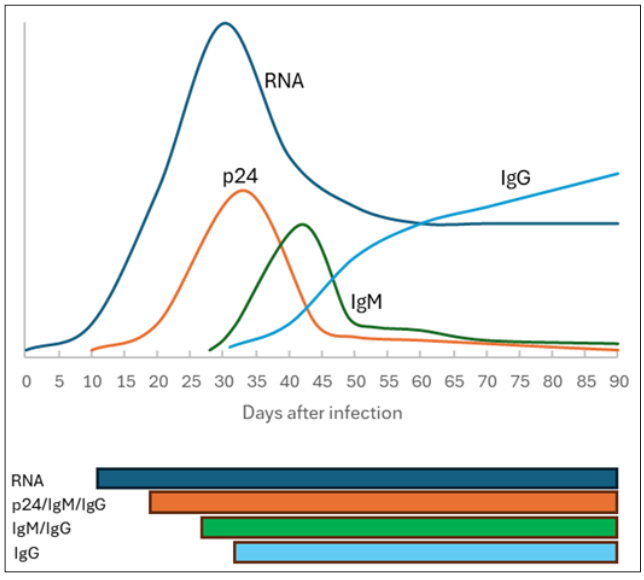

Figure 1. Time periods during which existing tests can detect HIV infection.

If the initial screening test is reactive, a supplemental antibody test with an FDA-approved HIV-1/HIV-2 antibody differentiation immunoassay should be conducted to confirm the presence of HIV infection and to differentiate between both types of HIV: HIV-1 and HIV-2. If the HIV-1/HIV-2 antibody differentiation immunoassay result is nonreactive (negative) or indeterminate (neither positive nor negative for HIV-1 or HIV-2), HIV-1 RNA testing should be conducted to detect the presence of HIV-1 RNA and confirm or exclude HIV-1 infection. These steps are critical for ensuring accurate diagnosis and appropriate clinical management (1). Time periods during which existing tests can detect HIV are marked on Figure 1.

HIV virion structure and the diagnostic roles of gp120, gp41, gp36, and p24/p26

The structure of the HIV particle is similar in both HIV-1 and HIV-2. It is roughly spherical, with a diameter of approximately 120 nm, and it is surrounded by a lipoprotein-rich membrane that is derived from the host cell.

Like other retroviruses, the HIV genome contains three major genes—*gag*, *env*, and *pol*—which encode the key structural and functional proteins of the virus (Figure 2).

- The *gag* gene encodes several internal structural proteins, most notably the capsid protein, p24, which forms the viral core and serves as an important early marker in HIV infection.

CLINICAL UTILITY

- **Screening and monitoring HIV infections in targeted populations to help curb the HIV epidemic**
- **Early detection and treatment to improve quality of life**

HIV-1 expresses the p24 antigen, while HIV-2 expresses a homologous protein that is commonly referred to as the p24 antigen, although it is also referred to in scientific literature as the HIV-2 p26 antigen.

- The *env* gene encodes the envelope glycoproteins:
  - In HIV-1, the *env* gene encodes the precursor gp160, which is cleaved into the envelope glycoproteins, gp120 and gp41, which are located on the surface of the virus. gp120 binds to CD4 receptors on host T cells, initiating viral entry, while gp41 anchors gp120 to the viral membrane and mediates fusion between the virus and host cell. Both gp120 and gp41 trigger strong antibody responses, which makes them ideal targets for HIV tests.
  - In HIV-2, the *env* gene encodes the precursor gp160, which is cleaved into the surface glycoprotein gp120 and the transmembrane glycoprotein gp36, the latter being functionally analogous to gp41 in HIV-1.
- Finally, the *pol* gene encodes enzymes that are essential for viral replication, including reverse transcriptase, integrase, and protease.

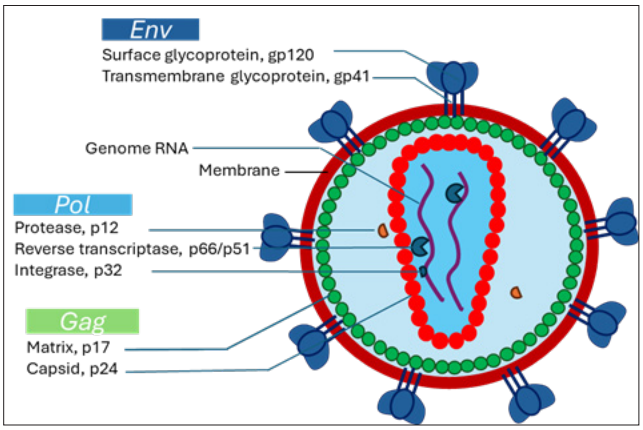

Figure 2. Scheme of mature HIV virion structure. Three main genes (*env, gag, pol*) encoding structural proteins and viral enzymes are also indicated in the figure.

HIV classification and geographic distribution

While HIV-1 is further divided into four groups—M, N, O, and P, group M is the most prevalent, and it includes multiple genetic subtypes (A, B, C, D, F, G, H, J, and K) as well as circulating recombinant forms (CRFs). The second significant group is group O (Outlier), although it accounts for approximately 1-2% of HIV-1 infections. The importance of this group is explained by the fact that in addition to Central Africa (the epicenter of origin) it was also found in some European countries, like France, Spain and Belgium. The sequence diversity between HIV-1 Group O and Group M strains is huge, reaching 50% and 30% in the *env* and *pol*, respectively. Due to the presence of the C181 mutation in Reverse transcriptase (RT) enzyme in Group O, more than 60% of individuals that live with this virus are faced with the challenge of drug resistance to some antiretroviral therapies (2). The diversity between group O and group M must be considered while developing new test systems and drugs, otherwise the diversity will hinder the diagnosis, monitoring, and treatment of Group O-infected patients. Groups N and P are extremely rare.

Meanwhile, HIV-2 comprises eight known groups that are designated from A through H, with groups A and B being the primary groups associated with widespread transmission. Conversely, groups C through H are considered rare (see Table 1).

Table 1.
HIV classification overview.

Virus Type	Group	Prevalence	Key Features	Geographic Distribution
HIV-1	M	Most prevalent	Contains multiple subtypes (A, B, C, D, F, G, H, J, K) and many CRFs (Circulating Recombinant Forms)	Global
	O	~1% of cases	Genetically distinct ("Outlier")	Central Africa and some European countries
	N	Extremely Rare	Limited circulation	Mainly in Cameroon
	P	Extremely Rare	Least characterized group	Very limited data
HIV-2	A&B	Most prevalent in HIV-2	Associated with widespread transmission	Mainly West Africa
	C-H	Rare	Limited transmission	Isolated cases, primarily in West Africa

Modern HIV immunoassays utilized for the initial screening need to be designed carefully due to virus genetic diversity. They should detect both HIV-1 group M subtypes, plenty of CRFs, HIV-1 group O strains and HIV-2 subtypes. For reliable HIV detection HIV combo immunoassays, targeting both p24 antigen and HIV-specific antibodies are recommended. Diagnostic performance of such assays is predominantly determined by the specificity and sensitivity of utilized p24-specific antibodies and the selection of proper HIV-specific proteins.

Early HIV detection markers and in Vitro diagnostic Regulation (IVDR) performance criteria for p24 antigen assays

HIV RNA can be detected 7–10 days after infection, while the p24 antigen in patient's blood can be detected 10–15 days post-infection, and HIV antibodies are typically detectable within two to eight weeks after infection (3), figure 1. The combined HIV p24 antigen and HIV antibody testing can shorten the diagnostic window period to approximately 14 days. According to the EU IVDR Class D regulatory requirements, the lower limit of detection (LoD) of p24 antigen immunoassays for the WHO HIV-1 p24 international standard (NIBSC code: 90/636) must be ≤ 2 IU/mL, and the assay specificity must be ≥ 99.5%. Therefore, the sensitivity and specificity of HIV p24 antigen assays are of critical importance.

HIV ASSAY DEVELOPMENT

P24 Monoclonal antibodies for HIV immunoassay development

Hytest offers several monoclonal antibodies (MAbs) specific to HIV p24, which can be used for the development of HIV p24 antigen immunoassays or HIV Ag/Ab combo assays based on a two-site sandwich format. All p24-specific antibodies were evaluated via sandwich chemiluminescent particle-based immunoassays (CLIA platform). Briefly, HIV p24-specific monoclonal antibodies are coated onto magnetic particles. These particles capture the p24 antigen from the serum sample. Then the captured p24 is detected using the second p24-specific monoclonal antibody, conjugated to Alkaline phosphatase (ALP). See figure 4A.

Sensitivity analysis on the CLIA platform using the WHO HIV-1 p24 international standard (NIBSC code: 90/636) indicates that assay prototypes utilizing new antibodies are able to achieve an analytical sensitivity of 0.5–0.64 IU/mL, which exceeds the requirements (≤ 2 IU/ml) set by the EU IVDR Class D regulations (4). The developed p24 assay prototypes demonstrate higher analytical sensitivity than p24 immunoassays from companies with the most extensive presence on the market. Details of the antibody combinations recommended for the assay development, using CLIA platform and their corresponding LoD data are summarized in Table 2. More data regarding possible antibody combinations recommended for the assay development are found in the section Ordering information. In addition, external clinical sample testing shows the specificity exceeds 99.95%, which meets clinical requirements.

Table 2.

LoDs for HIV p24 antigen assay prototypes.

Sample preparation: WHO HIV-1 p24 international standard material (NIBSC code: 90/636), HIV-1 p24 (group M) antigen, HIV-1 p24 (group O) antigen and HIV-2 p26 antigen.

Detection method: all of the samples were detected using sandwich-chemiluminescent immunoassay (alkaline phosphatase labelling).

The recommended assay prototypes exhibited a higher analytical sensitivity for HIV-1 p24 and HIV-2 p26 proteins than Abbott and Roche assays.

Capture MAb	Detection MAb	LoD of WHO international standard p24, IU/ml	LoD of HIV1 p24_M, pg/ml	LoD of HIV1 p24_O, pg/ml	LoD of HIV-2 p26, pg/ml
GA17	GA54	0.55	4	1.5	15
GA17	GA12	0.64	2	2	8
GA17	GA38	0.55	1.5	1.5	8
GA34	GA54	0.5	2	1.5	20
GA38	GA54	0.55	4	1.5	20
Abbott Alinity HIV Ag/Ab combo reagent kit		1.0	5	4	n/d when HIV-2 p26 is 1 ng/ml
Roche CombiPT HIV reagent kit		1.3	5	6	20

All of the recommended HIV p24 assay prototypes are capable of recognizing the WHO HIV-1 p24 international standard material (NIBSC code: 90/636), as well as the following HIV-1 subtypes: A1, B, C, D, F1/CRF12_BF/BF rec, G, CRF20_BG, CRF01_AE, CRF02_AG, H, and Group O (NIBSC code: 16/210). They also recognize the WHO International Reference reagent for HIV-2 p26 Antigen (NIBSC code: 16/236). These data indicate that the presented antibodies recognize shared epitopes (or conservative fragments) of non-identical p24 proteins (HIV-1 p24 group M, group O, p26 from HIV-2).

Ability of HIV p24 assay prototype GA12 (capture) - GA17 (detection) to recognize three p24 variants: in-house recombinant HIV-1 p24 group M, HIV-1 p24 group O and HIV-2 p26 used as calibrators is presented in Figure 3.

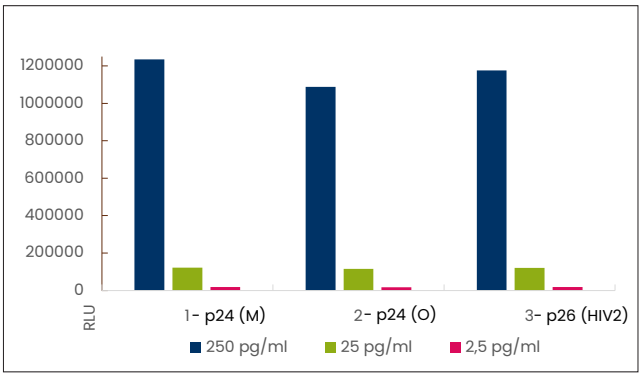

Figure 3. Representative results for p24 assay prototype GA12 (capture)-GA17 (detection) for detecting three p24 in-house recombinant variants (HIV-1 p24 group M, HIV-1 p24 group O, HIV-2 p26). Detection method: sandwich - chemiluminescent immunoassay (alkaline phosphatase labelling).

Recombinant proteins for HIV-1/HIV-2 IgM/IgG detection

Hytest also offers a panel of recombinant proteins for HIV-1/2 IgM/IgG detection. During infection, different antibodies appear at different times (5). Antibodies specific to gp41/gp36 are among the first to arise in HIV-1/HIV-2 infections, respectively. They are often detectable within 2–3 weeks post-infection, which means that gp41/gp36 is a valuable target for early diagnostic assays. In contrast, antibodies specific to gp120 (V3 loop) generally appear at a later stage, although the V3 region is highly immunogenic once exposed. To provide broad and reliable diagnostic coverage, Hytest offers the chimeric recombinant proteins which contain immunodominant regions of such proteins as HIV-1 gp41, gp120 (V3 loop), and HIV-2 gp36. These proteins were selected based on their high immunogenicity and early antibody response. To cover the maximal genetic diversity for HIV-1 gp41 group M protein the most common fragments for different gp41 strains of group M were used for protein engineering. The same approach was used for engineering gp120 V3 and gp36.

Cat. # 8H11, HIV-1, gp120-gp41 N-Trx, is a recombinant chimeric protein, expressed in procaryotic cells (E. coli). The protein is engineered by fusing a Thioredoxin with two Cys-to-Ser substitutions (C33S and C36S), HIV-1 V3 domain of gp120 from M-group, HIV-1 V3 domain of gp120 from O-group, ectodomain of gp41 from M-group, ectodomain fragment of gp41 from O-group and His10. Between the fragments (GS)2 or (GS)3 linkers were added. This design presents epitopes from both *env* subunits, with the aim of identifying antibodies of different specificity across HIV-1 strains. Thioredoxin tag was added to the N-terminus of the protein to facilitate solubility, when His10 tag was added for protein affinity purification.

Cat. # 8H12, HIV-1, gp41-gp120 N-Fc, is a recombinant chimeric protein expressed in a mammalian cell line. The protein is engineered by fusing an Fc-fragment (114-330 aar) of human IgG1, HIV-1 ectodomain fragment of gp41 from M-group, HIV-1 ectodomain fragment of gp41 from O-group, V3 domain of gp120 from M-group, and V3 domain of gp120 from O-group. Between the fragments $(GS)_2$ or $(GS)_3$ linkers were added. This design presents epitopes from both *env* subunits, with the aim of identifying antibodies of different specificity across HIV-1 strains. Fc-fragment was added to the N-terminus of the protein to facilitate solubility and further purification.

Cat. # 8H13, HIV-1, gp41 N-HSA, is a recombinant chimeric protein expressed in a mammalian cell line The protein is engineered by fusing Human serum albumin (1-609 aar), HIV-1 ectodomain fragment of gp41 from M-group and His10. Between HSA, gp41 fragment and His10 (GS)2 linkers were added. HSA-tag and His10-tag were added for protein solubility and protein purification, respectively.

Cat. # 8H16, HIV-1, gp120 C-Fc, is a recombinant chimeric protein expressed in a mammalian cell line. To address the high sequence diversity of gp120, the construct includes two fragments corresponding to the V3 region from Group M and Group O within a single fusion protein. The protein carries an Fc-fragment (114-330 aar) of human IgG1 at C-terminal to facilitate protein solubility and simplify protein purification.

Cat. # 8H24, HIV-2, gp36 N-HSA, is a recombinant chimeric protein expressed in a mammalian cell line. The protein is engineered by fusing human serum albumin (1-609 aar), HIV-2 ectodomain fragment of gp36, and His10. Between functional fragments $(GS)_2$ linkers were added. An N-terminal HAS-tag and C-terminal His-tag were used to facilitate solubility and protein purification, respectively.

Cat. # 8H25, HIV-2 gp36 C-TnC, is a recombinant chimeric protein expressed in a mammalian cell line. This protein is engineered by fusing HIV-2 ectodomain fragment of gp36 with human troponin C (1-161 aar), and His10. Between the functional fragments $(GS)_2$ linkers were added. This protein differs from Cat. # 8H24 in tag type and tag position; both recombinant proteins have the same amino acid sequence, corresponding to HIV-2 ectodomain fragment of gp36. The designs of Cat. # 8H24 and Cat. # 8H25 give assay developers flexibility across diagnostic formats and platforms, while supporting improved HIV-2 antibody detection.

Recombinant HIV-specific proteins, their short description and some of their physico-chemical properties (molecular weight, theoretical pI) are summarized in the table below (see Ordering information).

Hytest HIV Recombinant Proteins were evaluated on a CLIA Platform using two-site sandwich format. Briefly, HIV-1/2-specific recombinant proteins are coated onto magnetic particles. These particles capture the corresponding antibodies from the patient's serum sample. Then the captured antibodies are detected using Alkaline phosphatase (ALP)-conjugated HIV-1/2 -specific protein (see figure 4B).

Two selected HIV-1 antibody assay prototypes and four HIV-2 antibody assay prototypes were tested with their respective positive sample panels (11 samples in each panel) and demonstrated sensitivity of 100%.

Analytical specificity was assessed against various interference samples and clinical specimens. Two Hytest HIV-1 antibody assay prototypes met the performance requirement defined in Commission Implementing Regulation (EU) 2022/1107 (specificity >99.5%). Using the same specificity assessment approach, four Hytest HIV-2 antibody assay prototypes also met the (EU) 2022/1107 performance requirement (specificity >99.5%). See Table 3.

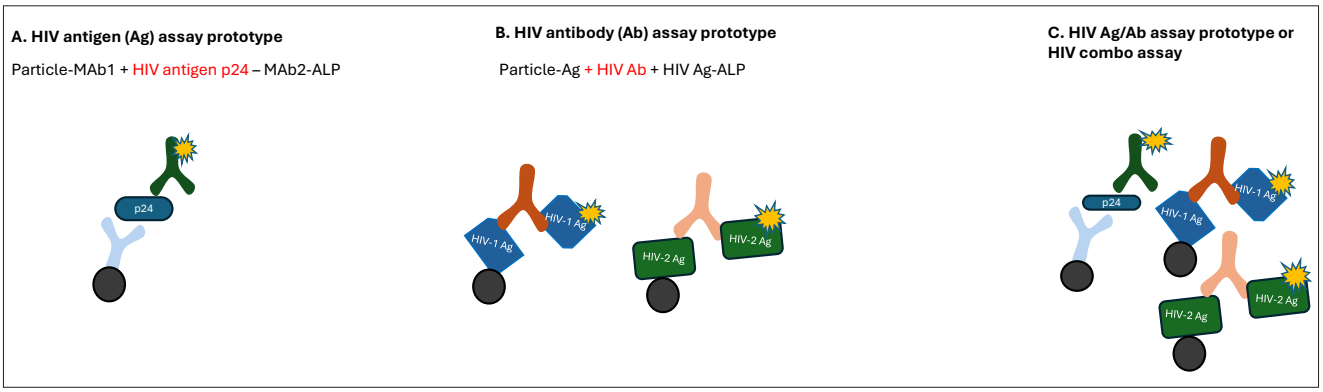

Figure 4.
The scheme of sandwich chemiluminescent particle-based HIV antigen (A), HIV antibody (B) and HIV Ag/Ab immunoassays (C) (CLIA platform).

Table 3.
Specificity evaluation of two HIV-1 and four HIV-2 antibody assay prototypes.

	HIV-1 antibody assay prototype		HIV-2 antibody assay prototype			
Particle Antigen, Cat.#	8H11	8H11	8H24	8H24	8H25	8H25
Conjugate Antigen, Cat.#	8H12	8H13	8H24	8H25	8H24	8H25
Interference sample number	305	305	78	268	78	78
False reactive	1	4	0	0	0	0
Clinical sample number	2957	5901	2652	6683	1638	750
False reactive	4	10	0	0	0	1
Total clinical samples specificity, %	99.85	99.77	100	100	100	99.88

Hytest HIV Ag /Ab Combo Assay Prototype Evaluation

Using the above-described combinations of p24 antigen and HIV antibody assay prototypes, two HIV combo assay prototypes were constructed and evaluated on a CLIA platform. HIV combo assay implies the use of HIV p24 antigen assay, HIV-1 antibody assay and HIV-2 antibody assay. However other or additional combinations of p24-specific antibodies for p24 detection and proteins for HIV antibody detection could be used for the Combo assay development. The scheme of HIV Ag /Ab Combo Assay in two-site sandwich format is presented in the Figure 4C. Both developed combo assay prototypes were able to detect the following tested genotypes: A, B, C, C/CRF_BC, C/CRF08_BC, C/CRF31_BC, CRF_BG, CRF01_AE, CRF02_AG, CRF03_AB, CRF22_01A1, D, F2, G.

For total of 4272 clinical samples with a variety of disease status, including interference samples, HIV combo assay prototype 1 showed a specificity of 99.93% and HIV combo assay prototype 2 showed 99.91% specificity (see Table 4).

Table 4.
Specificity evaluation of HIV Ag/Ab assay prototypes.

Assay	HIV Combo assay 1	HIV Combo assay 2
HIV-1 antibody assay	8H11 - 8H13	8H11 - 8H12
HIV-2 antibody assay	8H24 - 8H25	8H24 - 8H25
p24 antigen assay	GA17- GA12	GA17- GA54
Sample number	4272	4272
Number of false reactive	3	4
Specificity	99.93%	99.91%

REFERENCES

1. **Centers for Disease Control and Prevention and Association of Public Health Laboratories** (2018) Quick reference guide: recommended laboratory HIV testing algorithm for serum or plasma specimens.
2. **Bush S and Tebit DM** (2015) HIV-1 group O origin, evolution, pathogenesis, and treatment: unraveling the complexity of an outlier 25 years later. AIDS Rev. 17(3), 147-158.
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4. **European Commission** (2022) Commission Implementing Regulation (EU) 2022/1107 of 4 July 2022 laying down common specifications for certain class D in vitro diagnostic medical devices. Off J Eur Union. L 174, 3-42.
5. **Butler AL, Fischinger S, et al.** (2019) The antibodiome—mapping the humoral immune response to HIV. Curr HIV/AIDS Rep. 16(2), 169-179.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Origin	Isotype	Recommended combinations for sandwich immunoassays (capture-detection)
Monoclonal anti-HIV1/2 p24	3H24	GA12	mouse hybridoma	IgG1	GA17-GA12
					GA18-GA12
		GA15	mouse hybridoma	IgG1	GA15-GA18
					GA32-GA15
		GA17	mouse hybridoma	IgG1	GA17-GA12
					GA17-GA54
					GA17-GA38
		GA18	mouse hybridoma	IgG1	GA18-GA12
					GA32-GA18
		GA32	rabbit, recombinant	IgG	GA32-GA15
					GA32-GA18
		GA34	rabbit, recombinant	IgG	GA34-GA54
		GA38	rabbit, recombinant	IgG	GA38-GA54
		GA39	rabbit, recombinant	IgG	GA39-GA54
					GA17-GA39
					GA12-GA39
		GA54	rat, recombinant chimeric (with human IgG1 constant domain)	IgG1	GA17-GA54
					GA38-GA54

ANTIGENS

Product name	Cat. #	pI, theoretical	Molecular weight, kDa	Purity, %	Expression system
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp120-gp41 N-Trx), recombinant	8H11	5.93	56.98	≥80	bacterial cells
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp41-gp120 N-Fc), recombinant	8H12	6.55	66	>80	mammalian cells
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp41 N-HSA), recombinant	8H13	6.39	87	>80	mammalian cells
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp120 C-Fc), recombinant	8H16	8.34	33.3	>90	mammalian cells
Human Immunodeficiency Virus 2 Antigen (HIV-2, gp36 N-HSA), recombinant	8H24	6.5	86.6	>90	mammalian cells
Human Immunodeficiency Virus 2 Antigen (HIV-2, gp36 C-TnC), recombinant	8H25	4.7	37.6	>90	mammalian cells

NOTES

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Hytest

SCIENTIFIC EXCELLENCE FOR IVD

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